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OPHTHALMOLOGY

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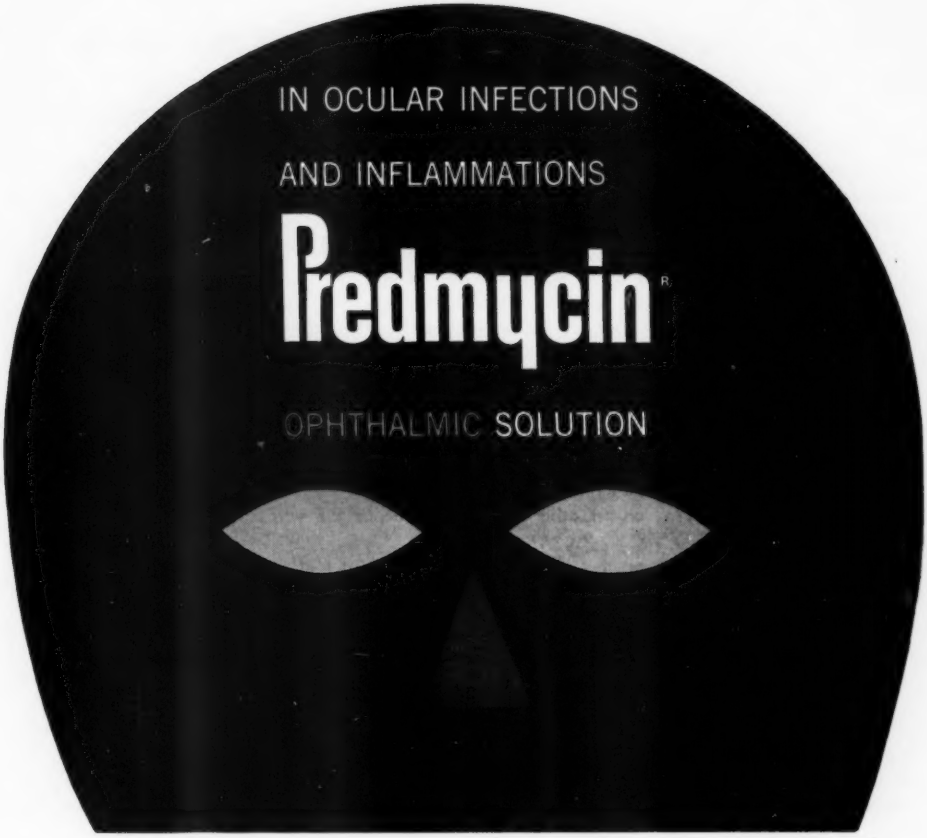

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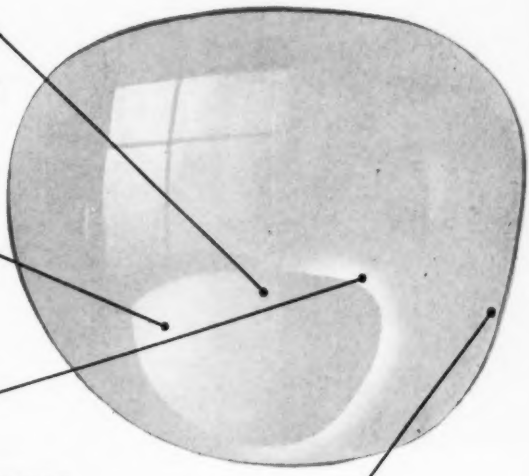

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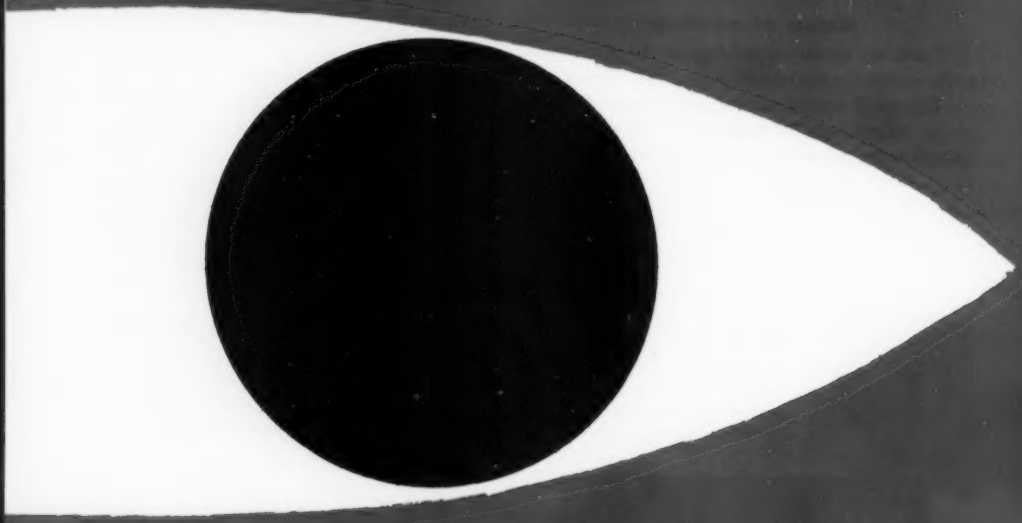

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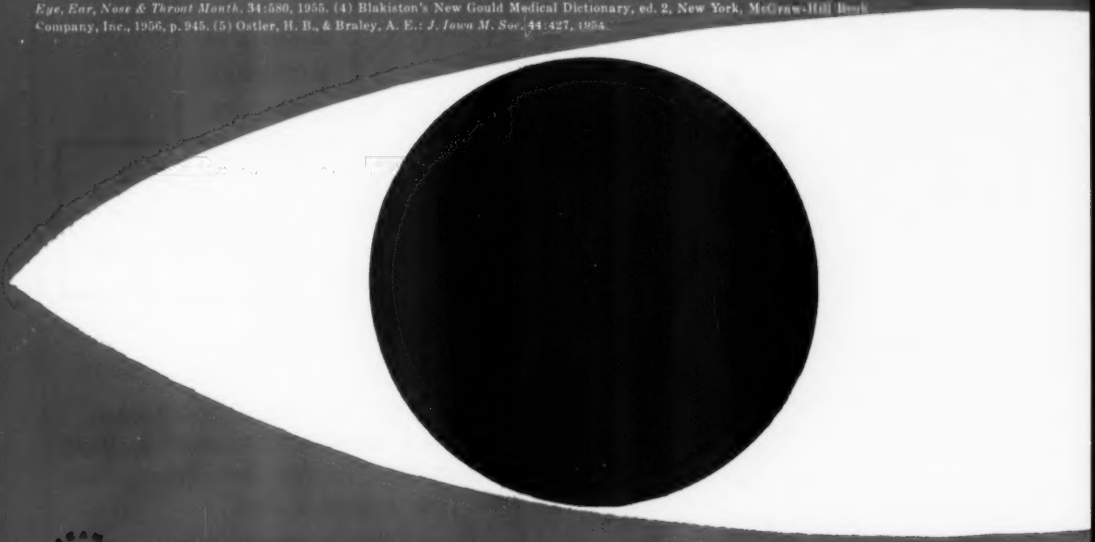

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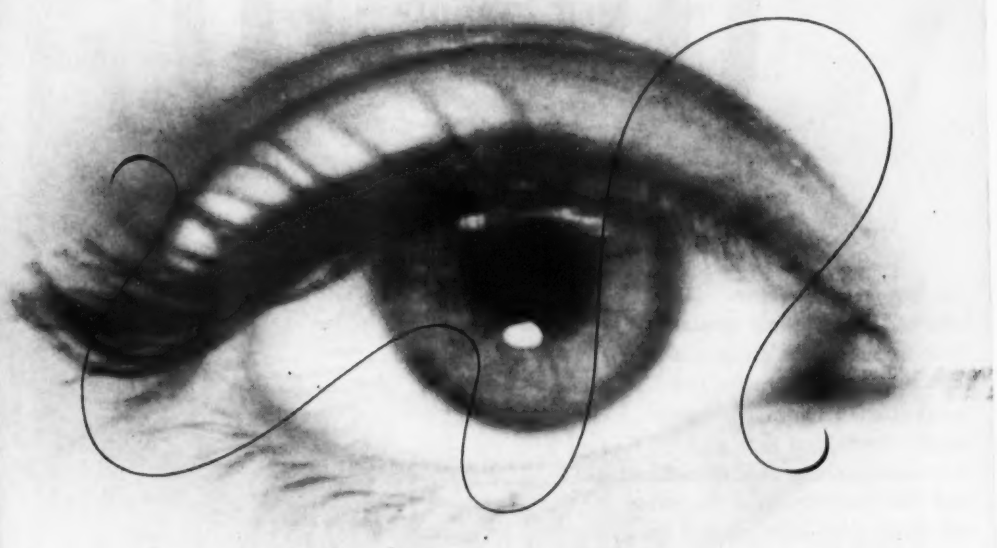

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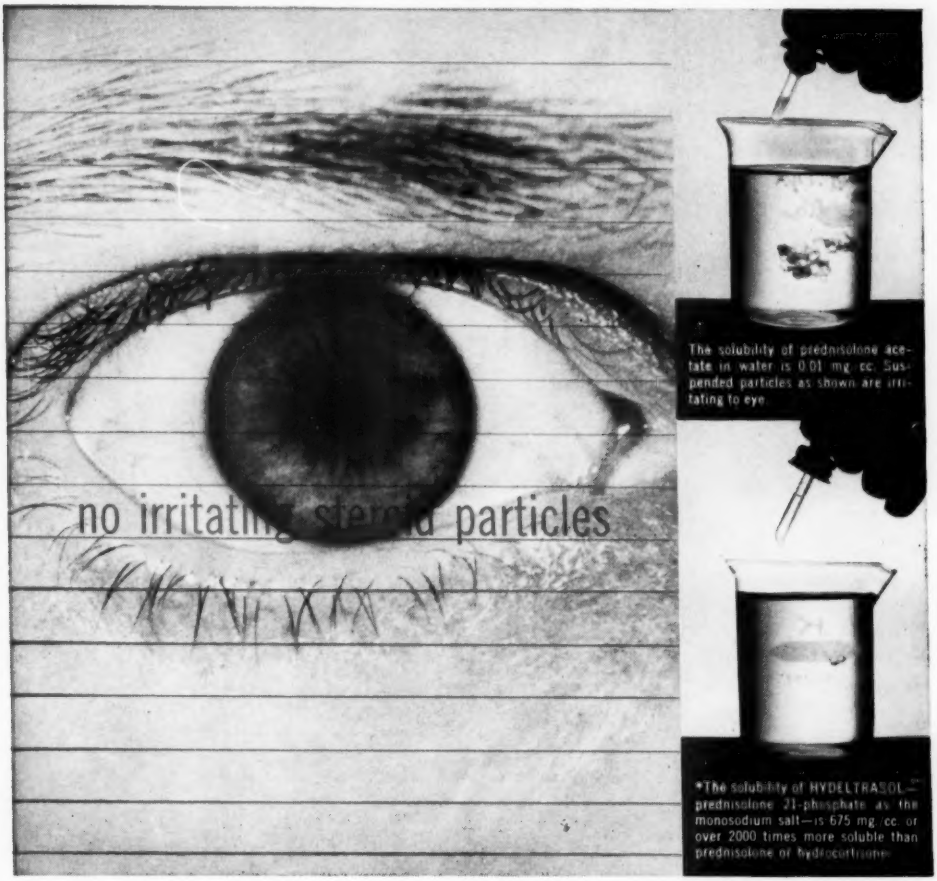
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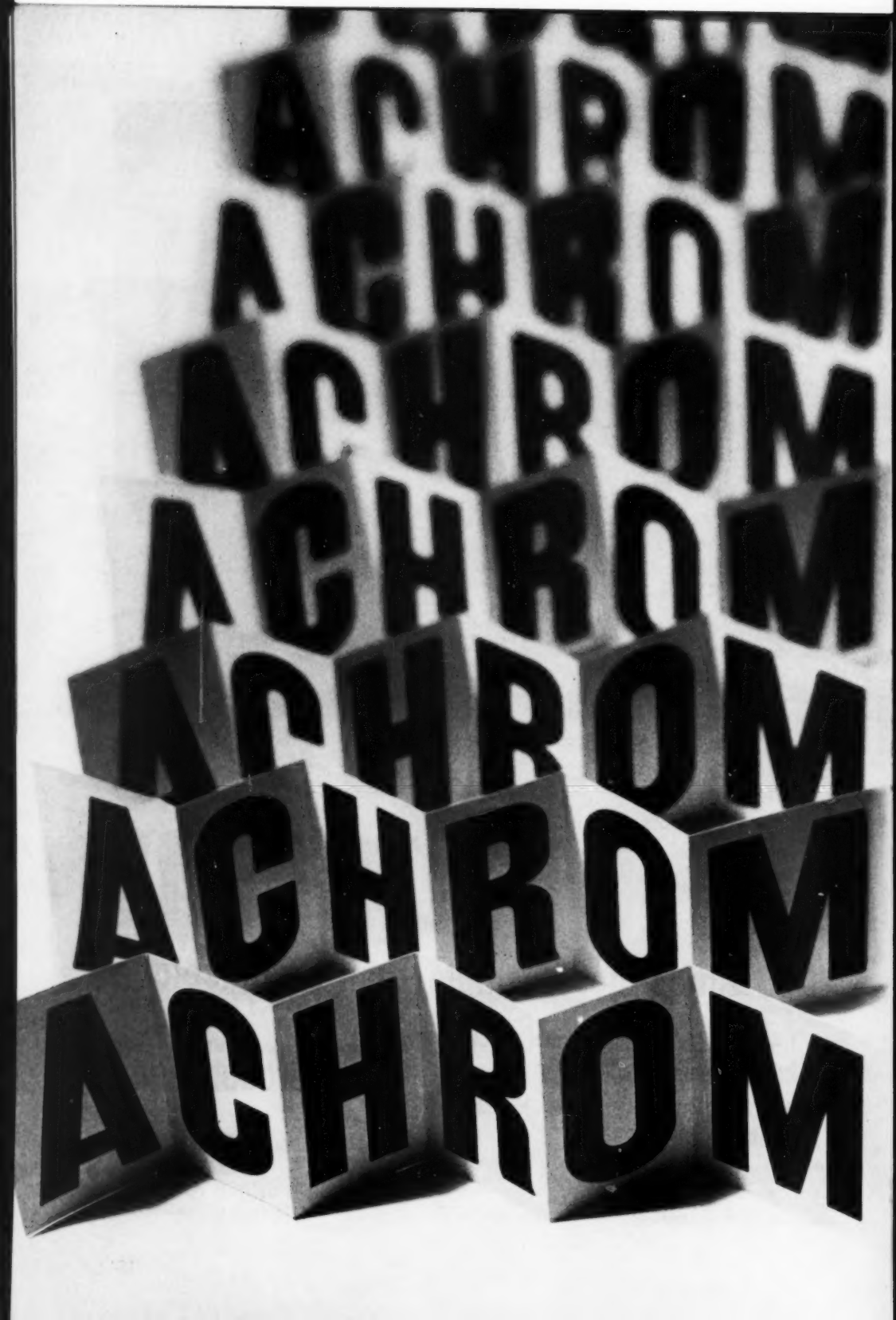
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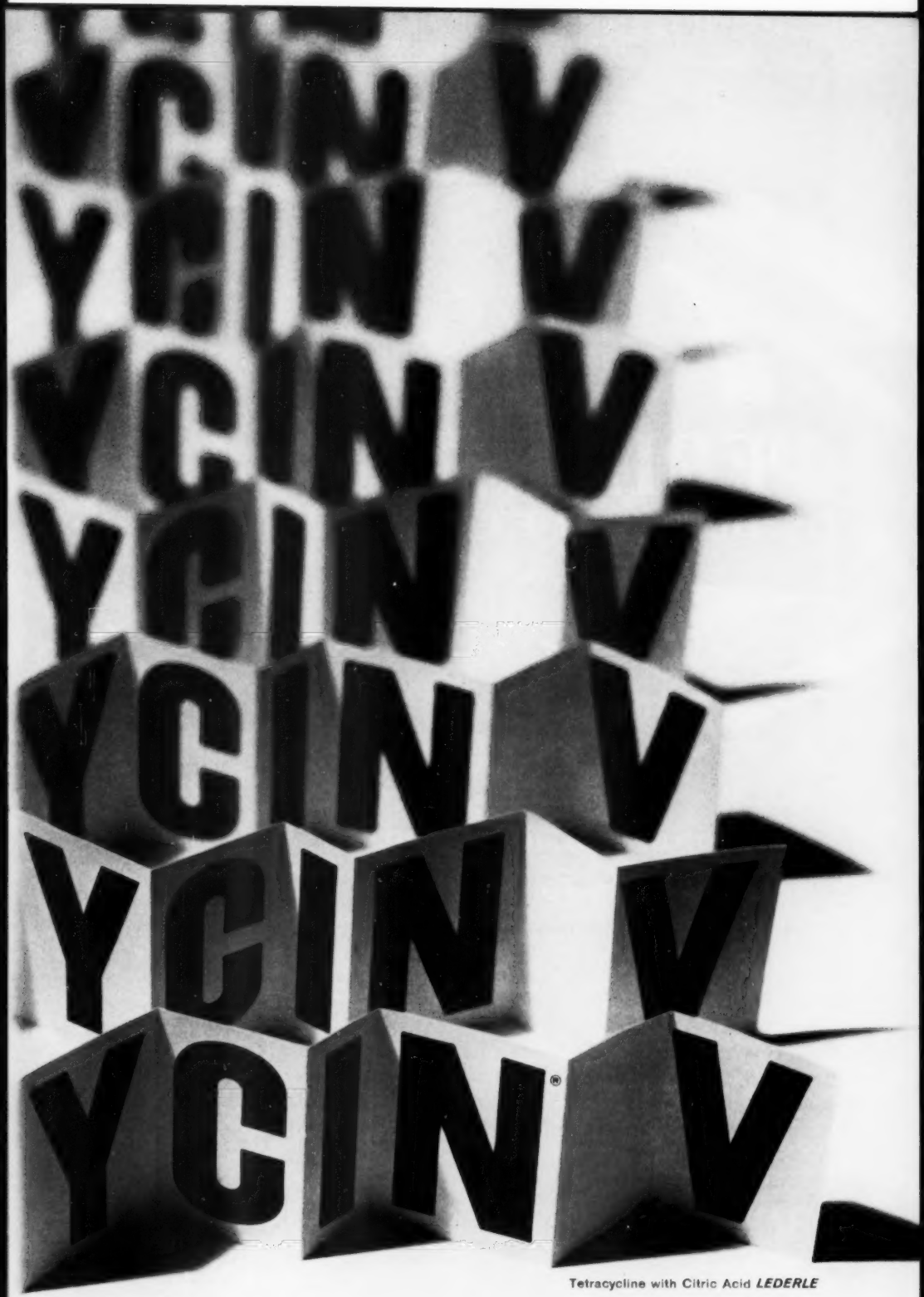

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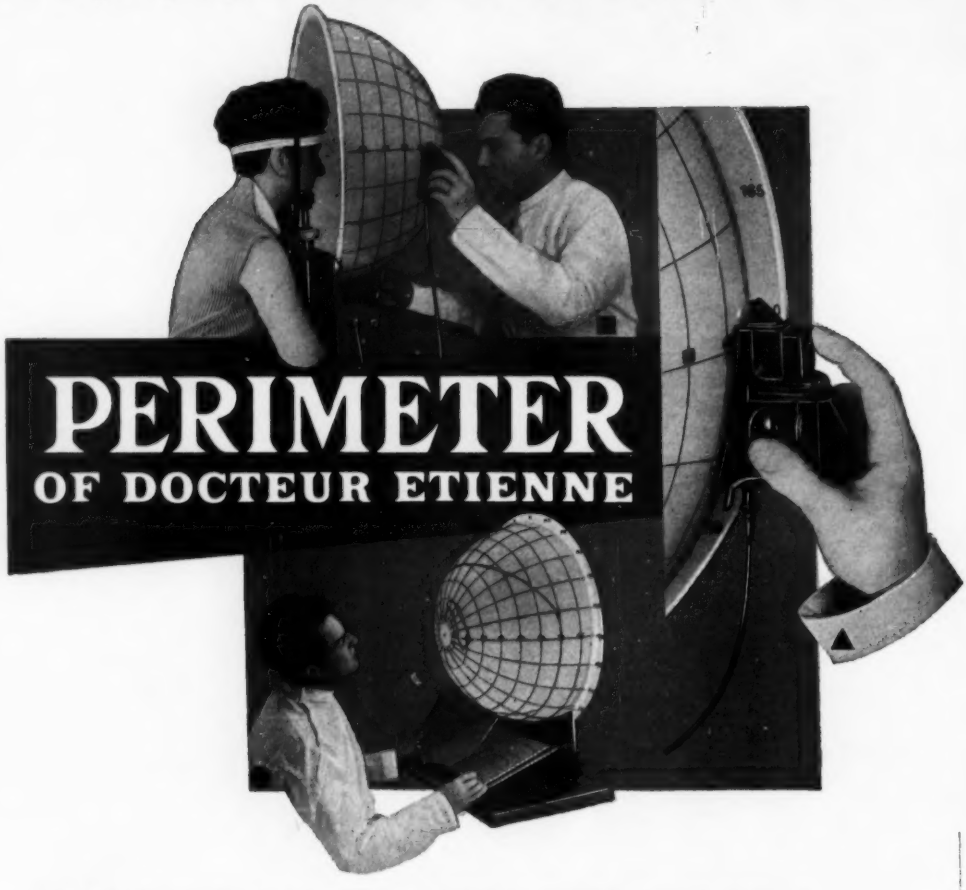

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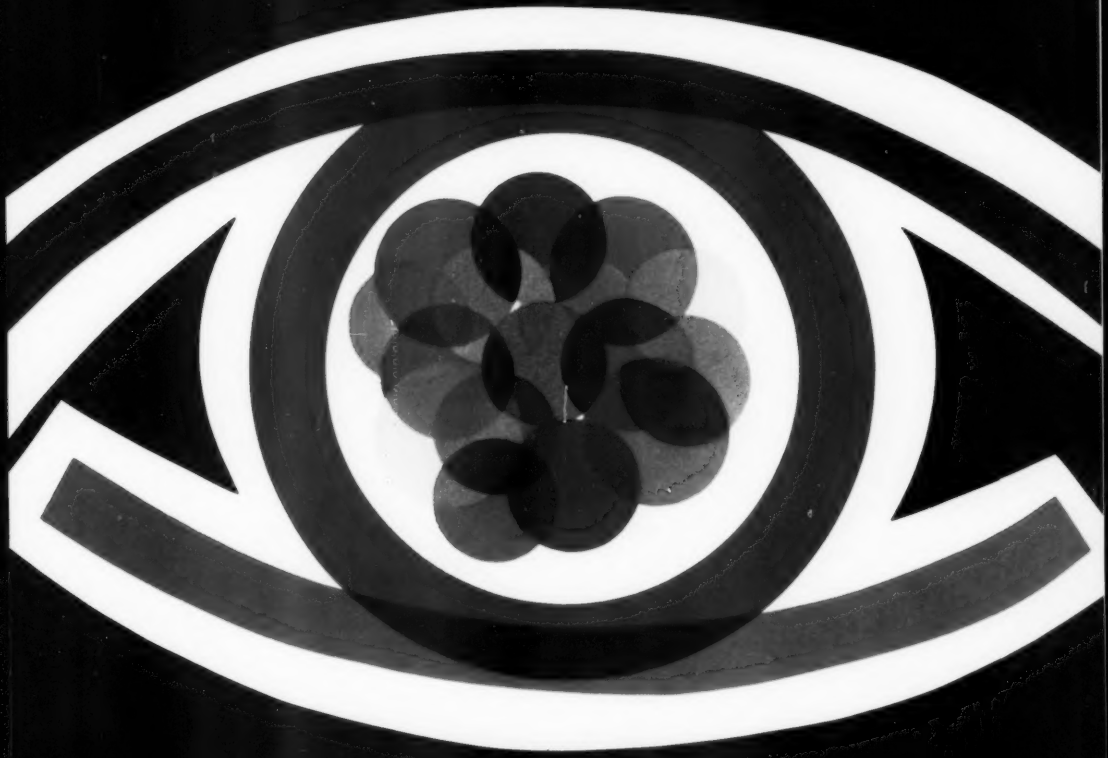

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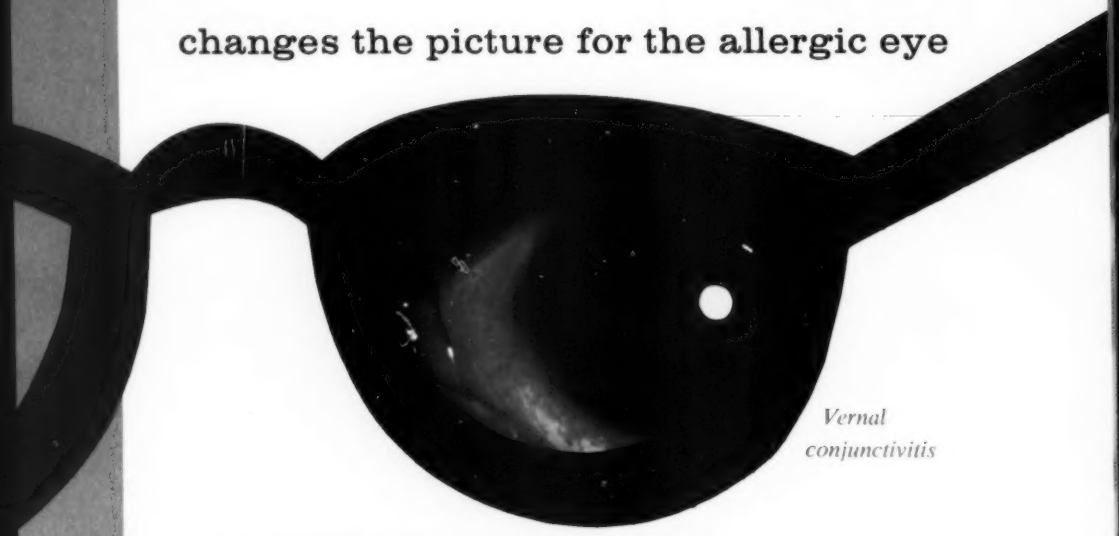

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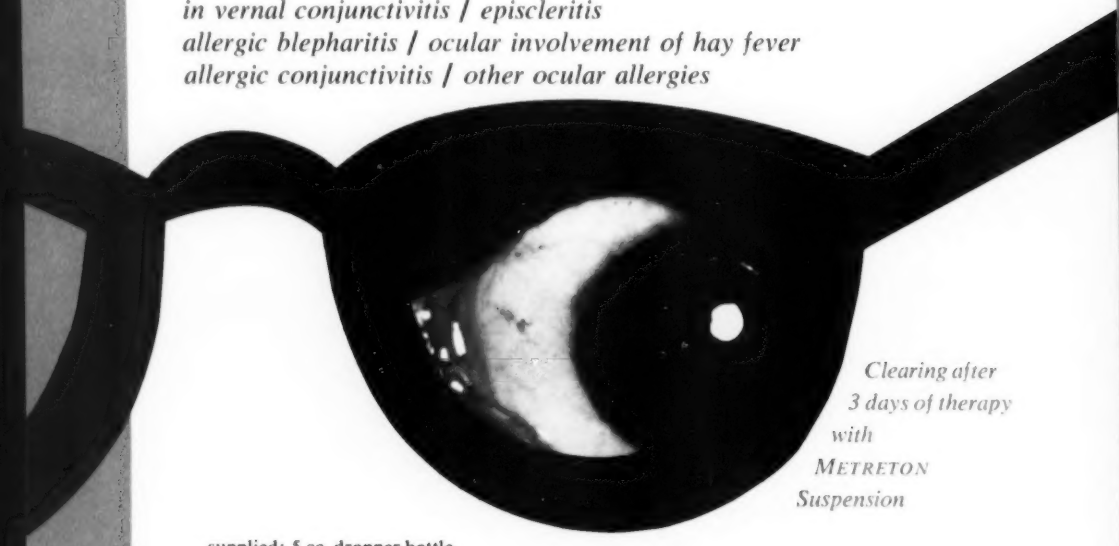

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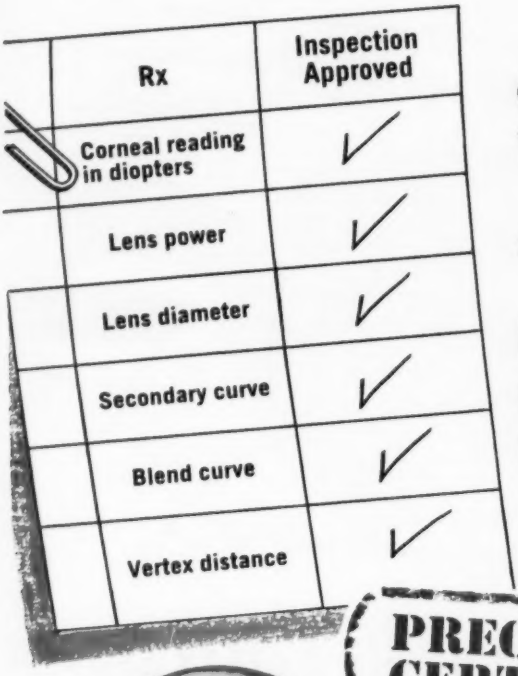

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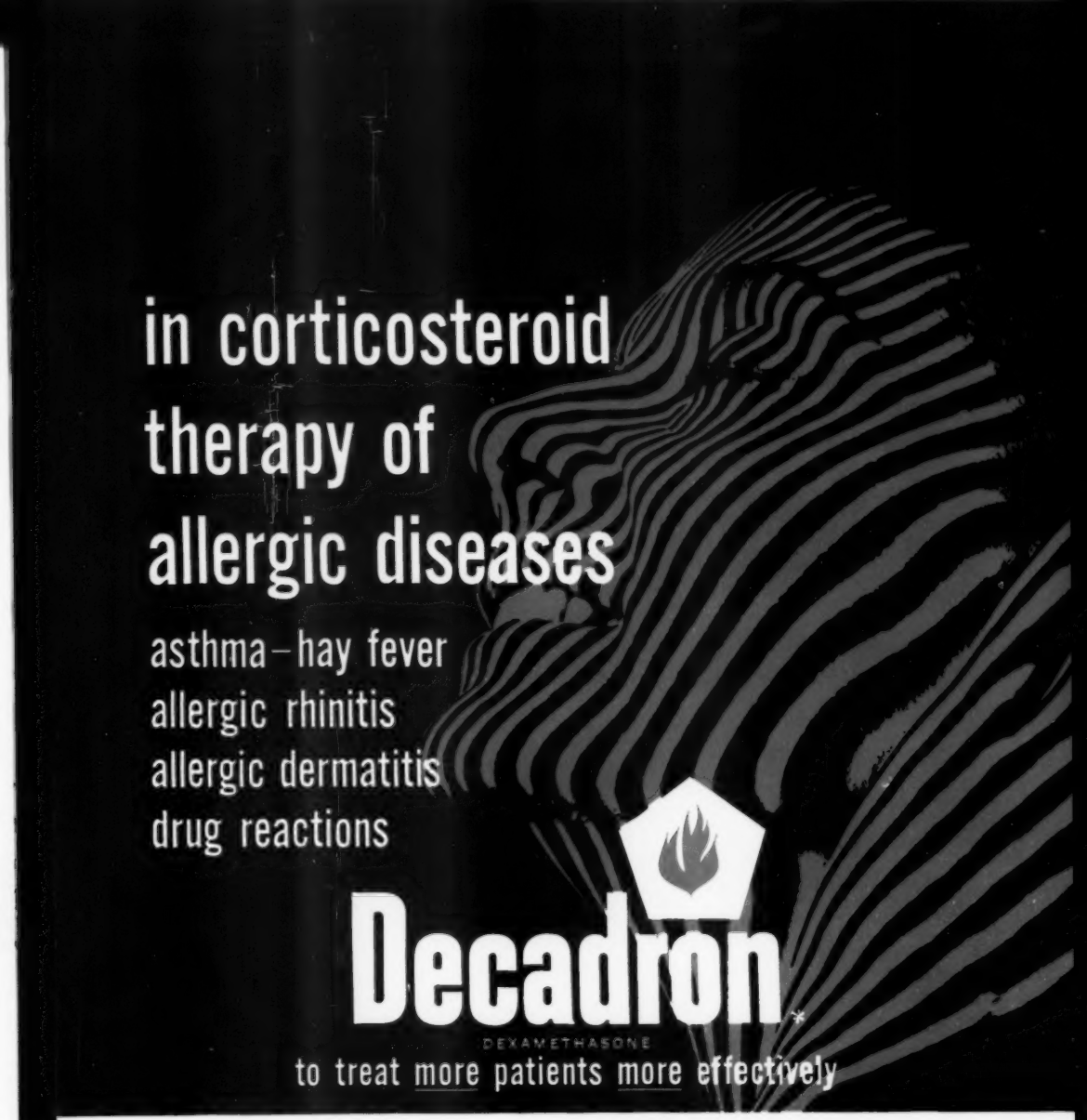

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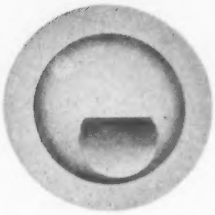

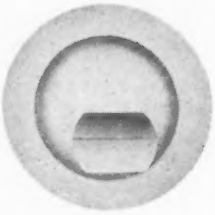

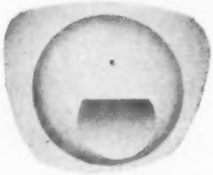

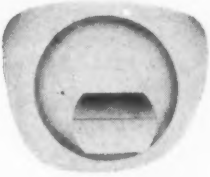

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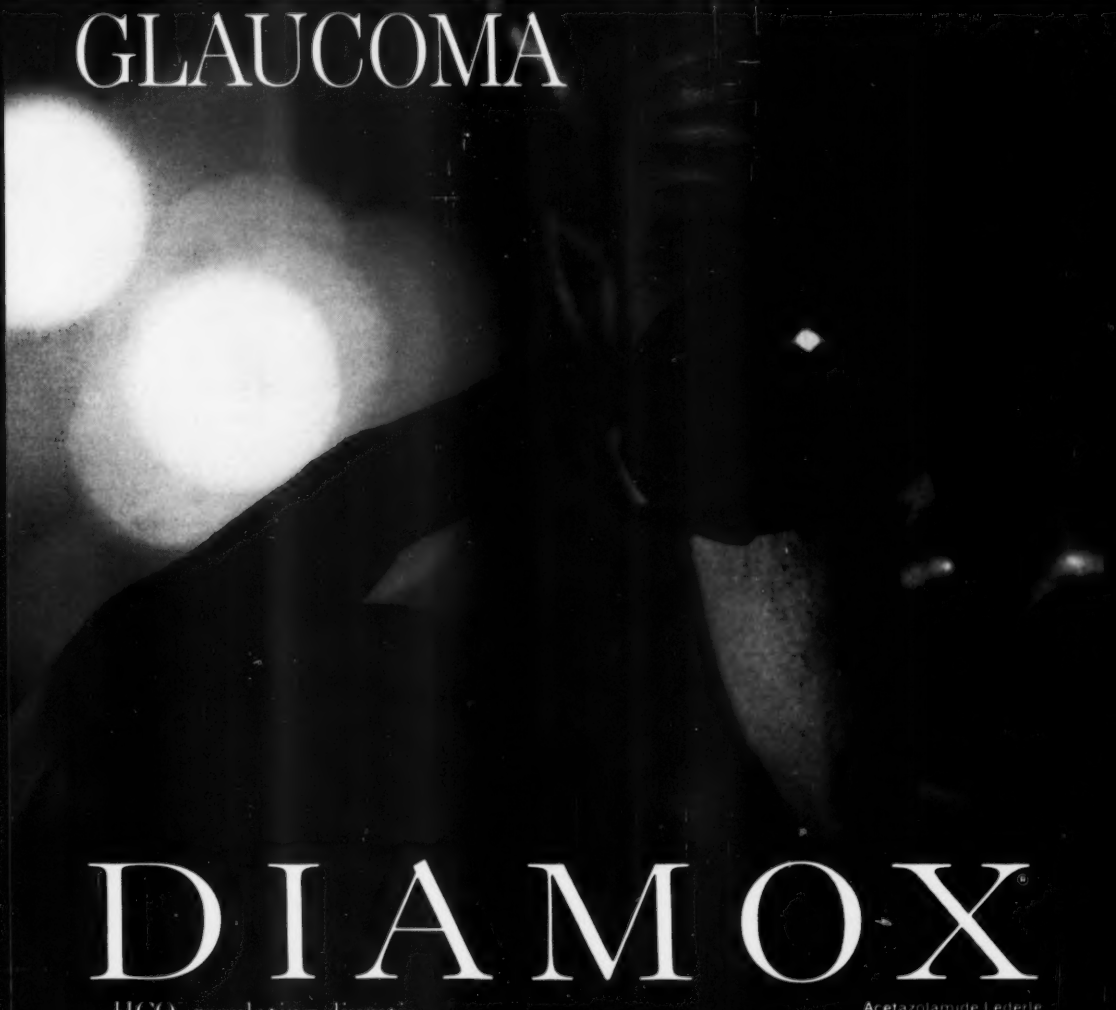
1. Venable, H. P.: J. Nat. M. A., 50:79, 1958.



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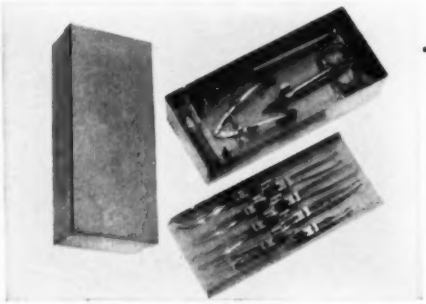

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WHEN AND HOW TO CORRECT VERTICAL IMBALANCE

We are all familiar with the characteristic complaints of a patient who has received bifocals, particularly first bifocals: without some form of compensation for vertical imbalance at the reading point—headaches after reading for only a short time, inability to fuse, suppression of one eye, etc. In order to avoid such complaints, we correct imbalances as low as 0.3 to 0.4 prism diopters. Whether correction for such slight amounts of vertical imbalance is necessary depends primarily on the use of the bifocal. You should correct this type of imbalance for an architect or an accountant but not for a salesman or housewife. In general, every imbalance over 1.00 prism diopter should be corrected. Perhaps because they are more frequent and more often overlooked, a surprising number of complaints arise from small uncorrected imbalances.

There are two instances when you should not correct the entire amount of induced vertical imbalance. First, when a bifocal wearer has accustomed himself to fusing with the imbalance; second, where a phoria exists which tends to balance the induced prism at the reading point.

THE FOUR METHODS OF CORRECTING VERTICAL IMBALANCE ARE:

1. **Drop Distance Centers** This divides the imbalance between the distance and reading portion. Whatever it removes from the reading portion of the lens it adds to the distance. For this reason it is used in imbalances of less than 0.6 prism diopter.

2. **Dissimilar Segments** We recommend this procedure only when two bifocal segments of similar nature are used, such as a combination of Ultex A and E, flat top D and R, Kryptok 23mm and 20mm. How much imbalance may be corrected in this manner is a function of the bifocal addition. Its practical limits are in the range of 1.25 prism diopters.

3. **Prism Segments** This is an extremely poor method because of the increased thickness of such a lens and its 1.50 prism diopter upper limit. It frequently is used for creation of horizontal prism in the segment only.

4. **Balance Center** This is the best method of correction for most imbalances. There is no upper limit but balance centers of less than 1.00 prism diopter are impractical to grind. Since it is always ground base up, the slab-off is used on the lens of the greatest minus power or the least plus power.

With some foresight in lens design, the ophthalmologist should never receive patient complaints about uncorrected or over-corrected vertical imbalance.

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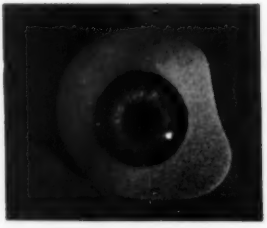

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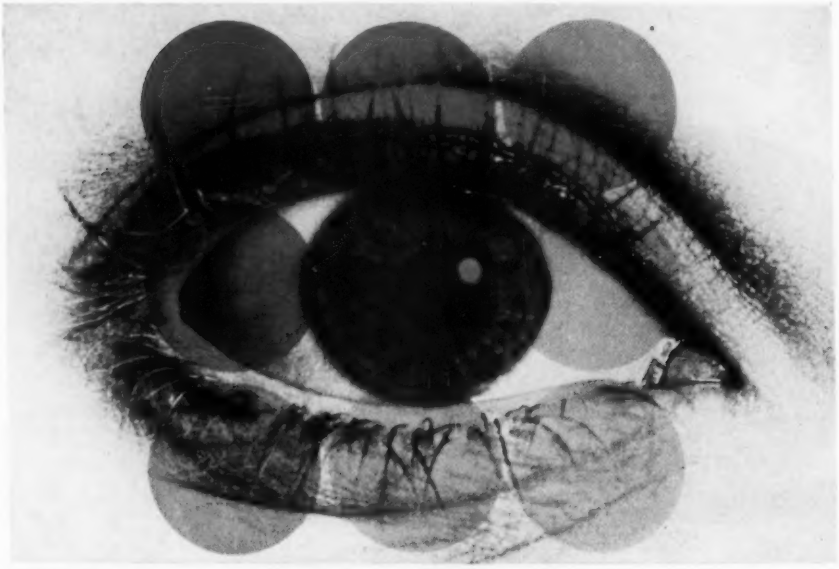

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1. New and Nonofficial Drugs; J. B. Lippincott Company, Philadelphia, 1958, p. 243.

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OPHTHALMOLOGY

Electric Response of the Phakic and Aphakic Human Eye to Stimulation with Near Ultraviolet

HERMANN M. BURIAN, M.D., Iowa City, and BENJAMIN ZIV, M.D., Boston

The spectral sensitivity of the human eye depends upon the sensitivity of the retinal elements. However, in the intact human eye the composition of the radiant energy reaching the retina is such that its sensitivity in the violet and near ultraviolet can not be evaluated fully by psychophysical methods. Owing to the absorption of the near ultraviolet by the yellow crystalline lens, which has a rather sharp cut-off at 400 mμ, ultraviolet radiation generally reaches the retina only in minimal quantities.

In one of his most remarkable papers, Wald [1] has shown, among other things, that with the removal of the lens the eye gains enormously in sensitivity in the violet and ultraviolet. Whereas in the intact human eye the sensitivity of the fovea declines at 365 mμ to $1/40{,}000$ of its maximal value at 562 mμ, aphakic rod-and-cone vision is still about $1/30$ as sensitive at 365 mμ as at the maximum, and the average sensitivity of the aphakic eye at 365 mμ is about 1,000 times that of the normal phakic eye. This increase in retinal sensitivity is expressed in the ability of the aphakic subject to read a Snellen chart when the illumination is confined to the region of 365 mμ, under which condition a phakic observer is unable to see the chart.

Submitted for publication Aug. 20, 1958.

Supported by Grant B-349 (C-5) of the National Institute of Neurological Diseases and Blindness.

From the Department of Ophthalmology, State University of Iowa College of Medicine. Clinical Fellow, The Retina Foundation, Boston (Dr. Ziv).

The difference in the response of the phakic and the aphakic human eye to ultraviolet stimulation has suggested to us the present investigation, which is concerned with the electroretinographic response of the phakic and aphakic eyes to ultraviolet stimulation.

Method

We used as the source of the ultraviolet radiation a 250 Watt AH5 GE mercury-

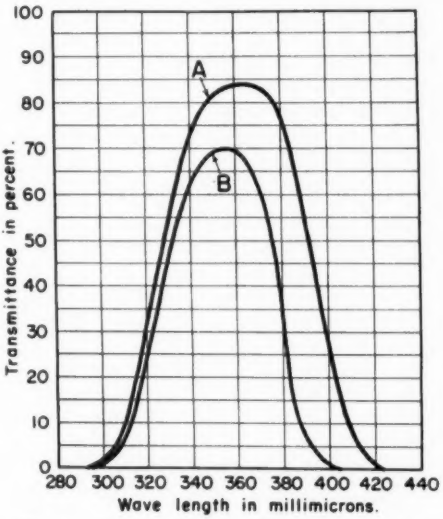

Fig. 1.—Characteristics of filters used. *A*, Corning filter No. 5970 (referred to in text as Filter 1). *B*, Corning filter No. 5840 (referred to in text as Filter 2). The third filter employed was a narrow-band-pass filter giving maximum transmission at approximately 365 mμ, obtained by combining Corning filters No. 7380 and 5860.

vapor lamp housed in a grounded air-cooled metal box and placed behind a round opening 4 cm. in diameter. The opening faced the eye to be examined and was provided with a Packard shutter, a photoelectric cell, and a holder for filters. Corning Filters No. 5970 (thickness, 4.5 mm.; Filter 1) and No. 5840 (thickness, 5.05 mm.; Filter 2) were employed. The characteristics of these filters are indicated in Figure 1. In addition, in all but one patient we also tested the eye with a narrow-band-pass filter composed of Corning Filters No. 7380 and 5860 combined together (Filter 3), giving a maximum transmission at about 365 mμ.

The eyes of the observers were prepared in the routine fashion adopted in our laboratory. The pupil was maximally dilated (7-8 mm.) with 2 drops of a 0.5% cyclopentolate (Cyclogyl) solution. Grounding of the patient was provided by Grass silver-cup electrodes fastened to each ear lobe of the patient. For the derivation of the retinal currents, the Burian-Allen speculum electrode [2] was employed, and a Grass silver cup electrode fastened to the supraorbital area served as reference electrode. All recordings were made with a 4-channel Grass ink-writing electroencephalograph.

The patient was seated comfortably in front of a metal bowl whose interior was painted with white nonglossy paint and which had an opening on the end opposite the patient, where the stimulating device was placed. The distance from the eye to the front of the metal box carrying the filters was approximately 35 cm. The patient's head was securely placed in a headrest, which had been adjusted in such a fashion that the eye was in line with a small red light source of adjustable intensity in the center of the opening of the metal box, serving as fixation point.

The eye to be examined was preadapted for 5 minutes to the light from two 60 Watt bulbs reflected from the interior of the bowl. The patient was then permitted to remain in complete darkness for 10 minutes. At the 10th minute of dark adaptation the eye was exposed to three or four flashes, with use of each of the three filters in succession. The Packard shutter was operated manually, and the exposure time for each flash was 0.8 to 1 second. The same procedure was repeated at the 20th and 30th minutes of dark adaptation. None of the patients wore spectacles during the procedure.

Results

Eight phakic subjects with 6/6 vision or better and normal eyes by slit-lamp, ophthalmoscopic, adaptometric, and perimetric examination, aged from 9 to 63 years, were studied. In selecting these observers special attention was paid to their lenses. None presented opacities of any kind. All gave excellent records with good base lines, with the exception of one 9-year-old girl, whose record had to be discarded.

The results obtained in the seven normal phakic observers whose records could be

TABLE 1.—*Amplitude of Responses of Normal Phakic Observers* *

			Dark Adaptation					
			20 Min.			30 Min.		
No.	Age, Yr.	Eye	Filter 1	Filter 2	Filter 3	Filter 1	Filter 2	Filter 3
1	9	O. S.	132	60	n. m. †	152	78	n. m. †
2	14	O. S.	72	40	n. m. †	92	80	n. m. †
3	20	O. S.	96	76	n. m. †	172	112	40
4	27	O. S.		No response				
5	39	O. S.		No response				
6	54	O. D.		No response				
7	63	O. D.		No response				

* In microvolts.
† Response not measurable.

evaluated are reported in Table 1. Each value represents the average of three individual electroretinograms. Only the 20- and 30-minute dark-adaptation levels are recorded, since none of the seven gave any response after 10 minutes of dark adaptation. Relatively good responses were obtained with Filters 1 and 2 in a 9-, 14-, and a 20-year-old observer. However, none of the observers 27 years old or older gave a response at any time.

These findings point up the fact that in children and young persons the pigmentation of the lens is still minimal. With advancing years this pigmentation increases to the point where so little ultraviolet penetrates to the retina that it provokes no electroretinographic response. Even so, the values obtained in children are low, and we recorded no electroretinogram with Filter 3, even after 30 minutes of dark adaptation, except in the 20-year-old observer, who generally gave relatively high responses. It is not surprising that some subjects give higher responses than others. The pigmentation of the lens is subject to considerable individual changes even within a narrow age group. Wald [1] has stated that in his normal subjects this factor alone added more than a logarithmic unit to the variation in sensitivity at 365 mμ.

All aphakic patients whom we tested showed the expected positive electroretinographic response to ultraviolet stimulation. We selected 10 patients who had had a cataract extraction. Our criteria for selection were as follows: round, freely reacting, and dilating pupil; absence of abnormalities detectable by slit-lamp or ophthalmoscopic examination, visual acuity of 6/9 or better. The age of the patients ranged from 58 to 84 years. Four of the records could not be measured on account of an unsteady base line or of eye movements occurring at the time of stimulation. It stands to reason that some of the elderly patients should not be able to cooperate sufficiently well to allow the recording of a good electroretinogram. Nevertheless, the sudden movements of the eyes at the time of stimulation indicated even in these patients that they perceived the light.

The results for the 20- and 30-minute adaptation levels of the six other aphakic patients are presented in Table 2. Here again, each value represents the average of three electroretinograms. It is evident from this Table that the retinas of all patients responded to the ultraviolet stimulation. Only two patients (No. 1 and 6) also gave responses at the 10-minute level. These responses were as follows: Filter 1: 148μv. and 104μv.; Filter 2: 108 and 80μv.; Filter 3: 76μv. and not measurable, for Patients No. 1 and No. 6, respectively. Patient No. 4 gave a response of 124μv. with Filter 1 at the 10-minute dark adaptation level but none with Filters 2 and 3.

It is of interest to note that these were the only responses which we obtained at the cone level of dark adaptation. This is probably explained by the fact that the electrical

TABLE 2.—*Amplitude of Response of Aphakic Patients* *

			Dark Adaptation					
			20 Min.			30 Min.		
No.	Age, Yr.	Eye	Filter 1	Filter 2	Filter 3	Filter 1	Filter 2	Filter 3
1	58	O. S.	192	188	160	260	220	164
2	67	O. D.	64	48	n. m. †	156	136	80
3	68	O. S.	184	148	128	244	216	168
4 ‡	74	O. D.	212	116	--	284	224	--
5	75	O. S.	96	88	72	140	144	92
6	78	O. D.	156	144	80	192	188	120

* In microvolts.
† Response not measurable.
‡ Filter 3 was not used in this patient.

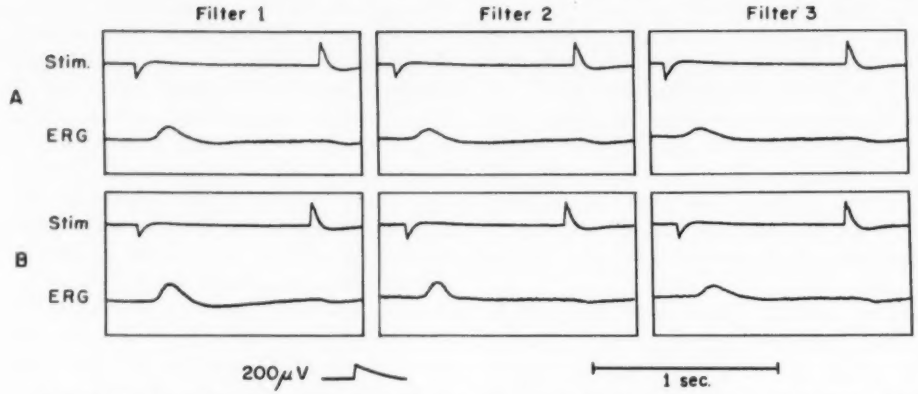

Fig. 2.—Sample electroretinograms from aphakic Patient No. 3. *A*, After 20 minutes of dark adaptation. *B*, After 30 minutes of dark adaptation. Markings in the upper channel of each record indicate onset and cessation of stimulation.

threshold of the retina, as determined by our present methods, is considerably higher than the psychophysical threshold. Had we used higher intensities of stimulation we might conceivably have also obtained cone responses.

Higher stimulus intensities might also influence the shape of the electroretinograms. The shape of all our retinograms (including those at the 10-minute level of dark adaptation) had essentially the character of rod responses: They consisted of slow protracted positive elevations (Fig. 2). No negative deflections were detected in any of the recordings, and the positive elevation was always a simple one. However, at the cessation of the stimulus there was a slight deflection, which was noted to appear very regularly. It would seem, therefore, that there is an off-response in the human electroretinogram with this type of stimulation. The peak time of the responses varied between 170 and 225 msec., being generally longer for the responses of lower amplitude than for those of higher amplitude.

Summary

A group of normal phakic subjects and a group of aphakic patients were examined electroretinographically, using near-ultraviolet radiation as the stimulus.

Phakic subjects 9 to 20 years of age responded with an electroretinogram, but none could be obtained from subjects 27 years or older. In contrast to this, all aphakic eyes gave electroretinograms with near-ultraviolet stimulation. The differences are explained by the presence or absence of a pigmented crystalline lens.

The electroretinograms consisted of slow protracted simple positive elevations.

NOTE: Since this paper was submitted for publication a paper by Dodt (*Nature* 181:286, 1958) came to our attention in which electroretinographic responses of two normal and two aphakic eyes to stimuli of various wavelengths, including near ultraviolet, are described. Dodt calculated the average selective spectral sensitivities from the relative number of quanta eliciting a b-wave of equal size. The results were compared with the curves for rod-vision given by Wald.[1]

University Hospitals, Department of Ophthalmology.

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Agar Microelectrophoresis at High Tension of Soluble Lens Proteins

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Hesselvik was the first to succeed, in 1939, in fractionating lenticular proteins by electrophoresis according to Tiselius. This method of investigation has since been used by numerous investigators studying the soluble proteins of the ocular lens, namely, Viollier, Labhart and Süllmann (1947), Smelzer and von Sallmann (1949), Lewis and Moses (1950), Bon (1955), Rao et al. (1955), Orekhovich (1957), and Bloemendal (1957).

Fractionation has subsequently been perfected with the introduction of paper electrophoresis (d'Ermo, 1952; Croisy, 1952; François, Wieme, Rabaey, and Neetens, 1953; Stemmerman, 1953; Bon, 1955), and it soon became apparent that the protein composition of the lens is more complex than would be suggested by the classical schema with its three crystallins (α-crystallin, β-crystallin, and albumin).

A comparative study of results obtained by electrophoresis has shown, among other things, that in a great many animal species the number of protein constituents of the lens exceeds the three fractions found in the electrophoretic diagram of the bovine lenticular proteins.

On the other hand, paper electrophoresis carried out under certain conditions (low ionic strength, increased voltage) has shown that Fractions II and III are by no means homogeneous either in bovines or in other animal species; these fractions seem to consist of several constituents.

Submitted for publication Aug. 19, 1958.
From the Ophthalmological Clinic of the University of Ghent, Prof. J. François, M. D., Director.

Whereas ordinary agar electrophoresis only reveals three fractions in bovines, the immunochemical methods (double diffusion in a gelified medium and immunoelectrophoresis) have shown that at least eight different antigens are to be found in lens extracts (François, Rabaey, Wieme and Kaminski, 1956).

After developing a technique of microelectrophoresis on agar (Wieme and Rabaey, 1957), we soon realized that this method offers very considerable advantages in the investigation of tissue proteins in general and lenticular proteins in particular.

One of the chief advantages of agar microelectrophoresis is the possibility of determining the mobility of the protein fractions under investigation. Although determination of the absolute mobility has yielded concordant results, mobility has been determined in the majority of cases as relative to human serum albumin (Rabaey, 1958). A solution of this albumin and dextran is deposited on the same sheet as the protein solution to be submitted to electrophoresis. For the sake of convenience, the mobility of the serum albumin is given the coefficient 1; the proteins under investigation are given a positive designation if they migrate toward the anode in relation to a point 0 (dextran band) or a negative designation if they migrate toward the cathode. The mobility of a protein fraction which migrates less far toward the anode than the serum albumin is expressed in a figure below 1, while the mobility of a fraction migrating farther than the serum albumin is expressed in a figure above 1.

The first results—obtained in lens extracts submitted to agar microelectrophoresis at

an ordinary temperature and a moderate potential gradient of the electric field—not only surprised us but were confusing. This is what we saw:

1. By this technique, the lens proteins in the majority of animals are divided into a considerably larger number of fractions than when submitted to paper electrophoresis. Five to ten different fractions were as a rule found.

2. Pherograms (electrophoretic diagrams) obtained with lenses from various animal species are hardly ever comparable, not only as to the number but also as to the distribution of the fractions obtained.

This wide variety in protein composition from one animal species to another is not limited to the lens proteins but also holds true for other tissues, as demonstrated by two investigations into the protein composition of the skeletal muscles in various species of fish (Connel, 1953; Nikkilä and Likno, 1955). Connel emphasizes the fact that electrophoretic diagrams show very marked differences between different species of fish, to an extent which might warrant a protein type of constant mobility, e. g., the statement that these diagrams are nearly as characteristic as fingerprints.

Obviously these marked differences between the species greatly impede investigations into tissue proteins.

The pherograms of the lenticular proteins are equally characteristic of the animal species examined, and it is no exaggeration to suppose that one day an animal will be recognizable by the electrophoretic diagram of its lenticular proteins.

A comparison of the mobility of fractions as directly shown in the pherograms has meanwhile shown that in mammals the situation is less confusing than was initially believed to be the case. In fact, it is obvious that fractions of established mobility are regularly encountered, particularly in the case of slow fractions (Rabaey, 1958).

By adjusting the evaporation of the cooling liquid (petroleum ether, as suggested by Wieme), electrophoresis can be made possible at any low temperature, a fact particularly useful for determination of mobility values.

Experiments at low temperatures (3-4 C) have yet another advantage: At these temperatures, there is less diffusion and spread of protein fractions during electrophoresis than at the usual temperature of 25 C; thus the fractions are narrower, more sharply delimited, and more distinctly separated.

It is true that the mobility of the fractions is reduced at lower temperatures, but this inconvenience can be overcome by increasing the potential gradient of the electric field. The development of heat is of no signifiance, as the heat produced in the thin sheet of agar gel is regularly and immediately removed by the surrounding petroleum ether.

Thus we have progressively increased the tension at which electrophoresis was carried out to 20, 40, and even 50 V/cm.

For this high-tension electrophoresis it is advisable to use very thin sheets of agar (0.6 to 1 mm.), lest the temperature gradient in the agar be too great.

Under these conditions the power of separation can be considerably increased. We were surprised to see that various bands obtained by agar electrophoresis at ordinary temperature and low or moderate potential gradient of the electric field, showing slight asymmetries hardly suggestive of heterogeneity, divided themselves into two or several fractions.

This technique is particularly useful in investigating fractions of intermediate mobility which not only are complex but also are apt to migrate in very close patterns.

The possibilities of agar microelectrophoresis at high tension are illustrated in the pherograms and corresponding diagrams of the lenticular proteins in Okapia johnstoni Sclater (okapi)* and in Scyllium canicula (spotted dogfish).†

* The Société Zoologique d'Anvers made available the okapi eyes.

† The living fish was made available to us by Mr. N. Fontaine.

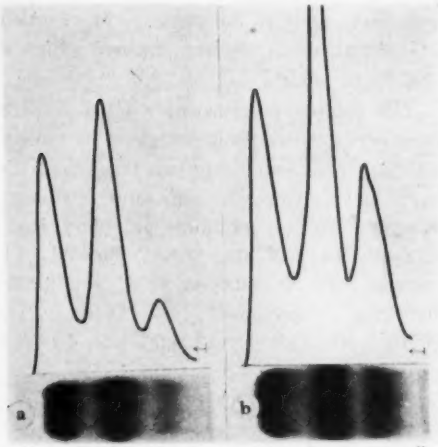

Fig. 1.—Paper pherogram and corresponding diagram of lenticular proteins in okapi; *A*, cortex. *B*, nucleus.

Paper electrophoresis (barbital [veronal] buffer solution; pH 8.6; ionic strength 0.1) revealed three fractions in the young okapi, both in total lenticular extracts and in cortical (Fig. 1*A*) and nuclear (Fig. 1*B*) extracts. The pherograms were generally the same as the bovine pherograms. The percentage of the various fractions is given in Table 1.

TABLE 1.—*Fractions After Paper Electrophoresis in Okapi*

	I	II	III
Cortex	35.2 *	52.9	11.9
Nucleus	28.1	47.1	24.8

* Figures indicate percentage.

In the same okapi, agar microelectrophoresis (barbital buffer solution; pH 8.4; ionic strength 0.03; temperature 4 C; potential gradient of the electric field 20 V/cm. for 25 minutes) revealed 11 fractions for the cortex and several fractions for the nucleus, as shown in Figures 2 and 3 and Table 2.

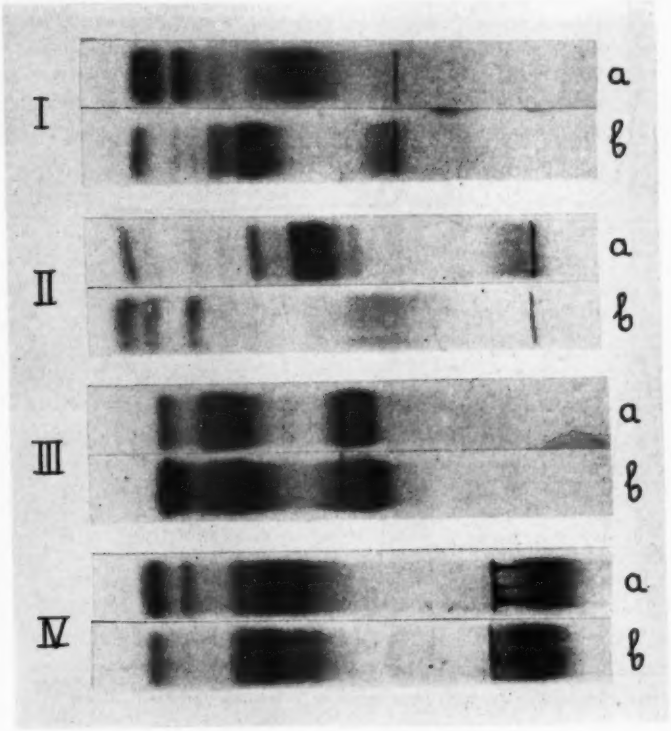

Fig. 2.—Agar micropherogram of lens proteins in spotted dogfish and okapi. *I*, Scyllium canicula: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes; (*a*) nucleus and (*b*) cortex. *II*. Scyllium canicula: temperature 4 C, potential gradient of electric field 40 V/cm. for 22 minutes; (*a*) cortex and (*b*) nucleus. *III*, Okapia johnstoni: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes; (*a*) cortex and (*b*) nucleus. *IV*, Okapia johnstoni: temperature 4 C, potential gradient of electric field 40 V/cm. for 22 minutes; (*a*) nucleus and (*b*) cortex.

TABLE 2.—*Agar Microelectrophoresis of Soluble Lenticular Proteins in Okapi*

Cortex		Nucleus	
Relative Mobility of Fractions	% of Fractions	Relative Mobility of Fractions	% of Fractions
1.5 ⇄1.1 (rapid fractions)	4.6	1.5 ⇄1.1 (rapid fractions)	1.3
0.94 (α-crystallin)	43.3	0.97 (α-crystallin)	37.0
0.64	1.9	0.64 ⇄0.24 (max. 0.38)	45.6
0.55	2.6		
0.45	10.3		
0.38	21.0		
0.32 ⇄0.29	7.2		
0.23	1.5		
0.18	1.9	0.18	4.9
0.11	5.5	0.11	11.0
0.01	0.2		

Agar microelectrophoresis at high tension (barbital buffer solution; pH 8.4; ionic strength 0.03; temperature 4 C; potential gradient of the electric field 40 V/cm. for 22 minutes), again in the same okapi, revealed 16 cortical and 17 nuclear fractions (Figs. 2 and 4 and Table 3).

The soluble proteins in spotted dogfish have been previously investigated by Croisy (1952), who found only two fractions with paper electrophoresis. The same technique (barbital buffer solution; pH 8.6; ionic strength 0.1), as used by us, showed (1) cortical part—5 fractions (Fig. 5), in the following percentages: I, 14.8%; II, 48.0%; III, 11.4%; IV, 6.0%; V, 19.8%; (2) nuclear part—2 fractions (Fig. 5), 34.7% and 65.3%, respectively.

Agar microelectrophoresis (barbital buffer solution; pH 8.4; ionic strength 0.03; temperature 4 C; potential gradient of the electric field 20 V/cm. for 25 minutes) in the same fish revealed 11 cortical and 12

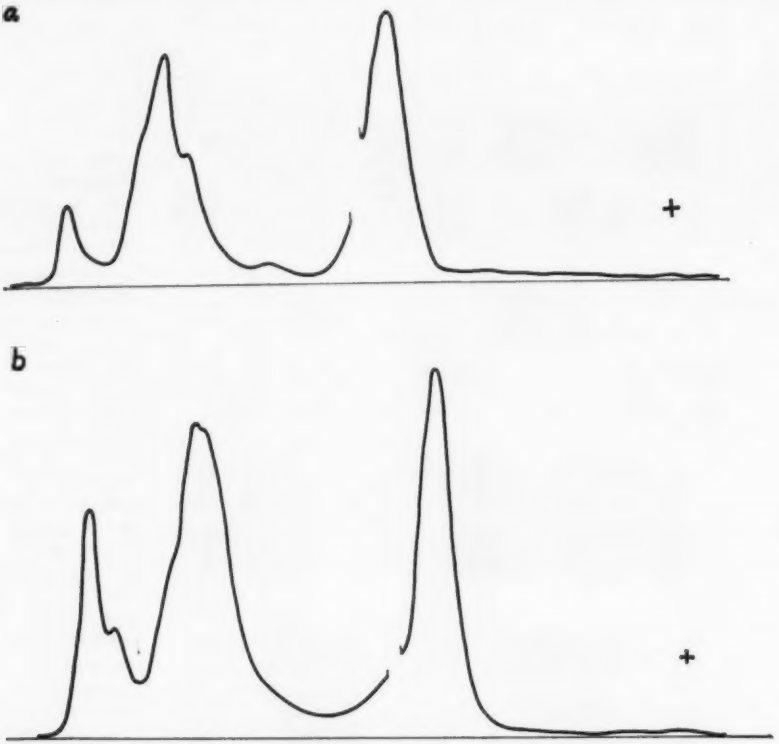

Fig. 3.—Diagrams of agar micropherograms of lens proteins in Okapia johnstoni: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes; (*a*) cortex and (*b*) nucleus.

TABLE 3.—*Agar Microelectrophoresis at High Tension of Soluble Lenticular Proteins in Okapi* *

Cortex		Nucleus	
Relative Mobility of Fractions	% of Fractions	Relative Mobility of Fractions	% of Fractions
0.94 (α-crystallin)	37.4	0.97 (α-crystallin)	31.9
0.64	13.0	0.67 ⇄ 0.59	2.0
0.58	0.8	0.59 ⇄ 0.55	1.5
0.55	1.0	0.54	1.3
0.52	1.3	0.51	2.0
0.50	1.9	0.49	1.8
0.47	3.3	0.46	3.2
0.44	6.6	0.43	7.9
0.42 ⇄ 0.36 (max. 0.38)	16.7	0.40 ⇄ 0.37 (max. 0.38)	10.7
0.31	9.1	0.30	7.5
0.24	2.2	0.24	2.5
0.19	2.1	0.18	5.3
0.15	1.4	0.14	2.4
0.11	4.4	0.11	7.3
0.09	0.6	0.09	2.6
		0.04	0.2

* Rapid fractions not taken into account.

nuclear fractions (Figs. 2 and 6 and Table 4.)

Agar microelectrophoresis at high tension (barbital buffer solution pH 8.4; ionic strength 0.03; temperature 4 C; potential gradient of the electric field 40 V/cm. for 22 minutes), again in the same fish, revealed

TABLE 4.—*Agar Microelectrophoresis of Soluble Lenticular Proteins in Spotted Dogfish*

Cortex		Nucleus	
Relative Mobility of Fractions	% of Fractions	Relative Mobility of Fractions	% of Fractions
1.60 ⇄ 1.04 (rapid fractions)	2.3	1.04 (rapid fractions)	2.0
0.81 (α-crystallin)	11.1	0.81 (α-crystallin)	2.9
0.51 ⇄ 0.42	4.9	0.67 ⇄ 0.46 (max. 0.48)	22.1
		0.46 ⇄ 0.37 (max. 0.44)	11.6
0.36	31.5	0.34	3.1
0.30	23.9	0.30	1.6
0.24	4.8	0.25	1.9
0.20	8.5	0.20	3.3
0.12	2.0	0.12	4.9
0.07	2.3	0.07	13.6
−0.03	2.1	−0.03	15.6
−0.08	8.6	−0.08	17.4

16 cortical and 15 nuclear fractions (Figs. 2 and 7 and Table 5).

Paper electrophoresis of lenticular proteins in infants reveals four different fractions, Fraction I corresponding with α-crystallin. By agar electrophoresis at 25 C, using relatively low tension (8-10 V/cm.), we were able to distinguish at least 10 different fractions (François and Rabaey, 1957).

Agar microelectrophoresis at high tension and low temperature further increases the

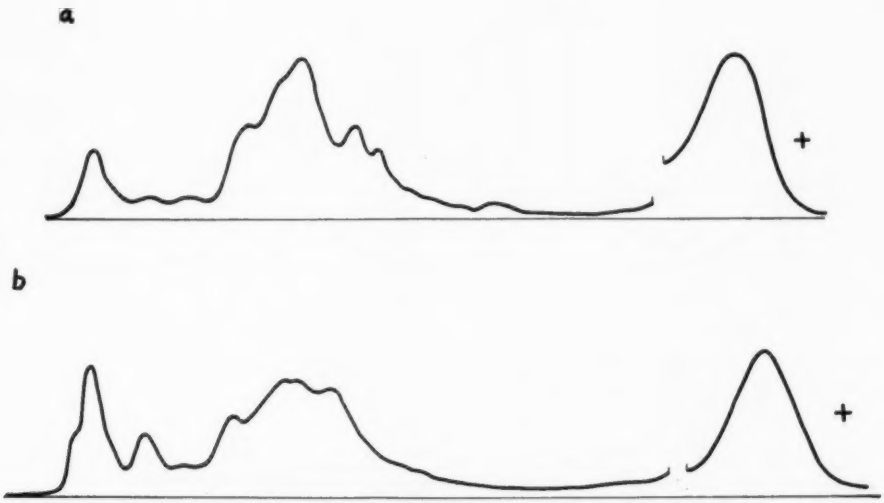

Fig. 4.—Diagrams of agar microphérograms of lens proteins in Okapia johnstoni: temperature 4 C, potential gradient of electric field 40 V/cm. for 22 minutes (rapid fractions not taken into account); (*a*) cortex and (*b*) nucleus.

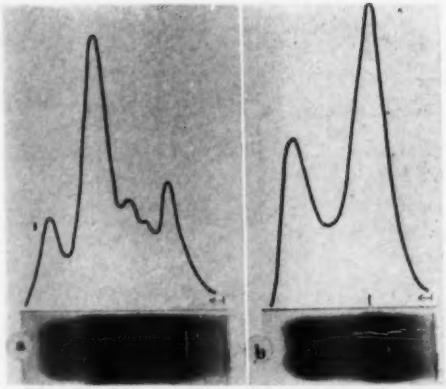

Fig. 5.—Paper pherogram and corresponding diagram of lens proteins in spotted dogfish; (*a*) cortex and (*b*) nucleus.

power of separation and makes 13 fractions distinguishable with certainty.

For comparison we present pherograms and diagrams of soluble lenticular proteins in a neonate, first as fractionated by paper electrophoresis (Fig. 8) and then as fractionated by agar electrophoresis at 4 C and an electric field potential of 20 V/cm. for 25 minutes (Figs. 9 and 10). Tables 6, 7, and 8 show the proportion of the various fractions and their mobility according to

TABLE 5.—*Agar Microelectrophoresis at High Tension of Soluble Lenticular Proteins in Spotted Dogfish* *

Cortex		Nucleus	
Relative Mobility of Fractions	% of Fractions	Relative Mobility of Fractions	% of Fractions
0.81 (α-crystallin)	16.5	α-crystallin	—
0.54	2.1	0.61 ⇄ 0.52	14.7
0.49	1.4	0.49	6.5
0.44	4.4	0.45	7.9
0.42 ⇄ 0.36	17.6	0.42 ⇄ 0.37	5.0
0.34	15.6	0.35	3.5
0.30	13.8	0.30	1.3
0.25	3.6	0.26	2.5
0.20	9.0	0.20	3.6
0.16	1.1	0.16	2.2
0.13	1.7	0.13	3.6
0.07	2.7	0.07	14.5
0.03	0.5	0.02	1.3
−0.03	2.5	−0.02	14.3
−0.08	7.3	−0.08	19.1

* Rapid fractions not taken into account.

paper electrophoresis, agar electrophoresis at 25 C (10 V/cm. for 25 minutes) and at 4 C (20 V/cm. for 25 minutes).

The protein composition of the human lens rapidly changes during aging (François and Rabaey, 1957). Even with agar electrophoresis at ordinary temperatures and low tension, without determination of mobility,

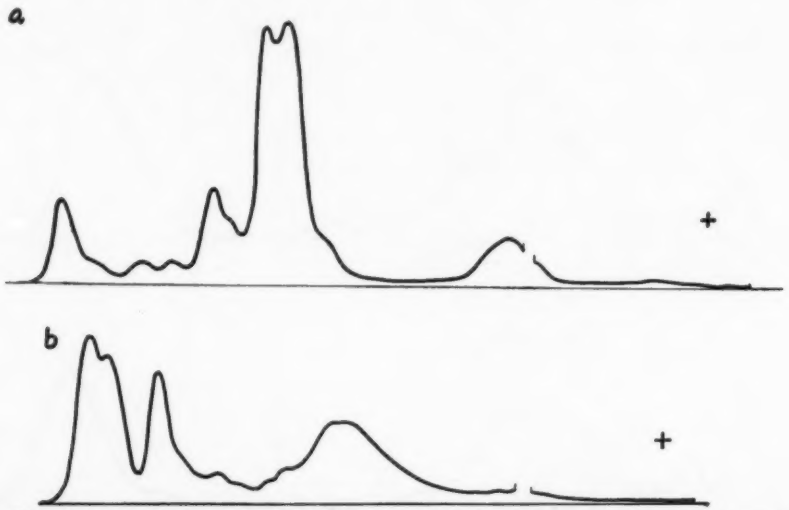

Fig. 6.—Diagrams of agar microphérograms of lens proteins in Scyllium canicula: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes; (*a*) cortex and (*b*) nucleus. The place where the line is interrupted corresponds to the place where the solution has been introduced into the agar gel.

TABLE 6.—*Paper Electrophoresis of the Lens of a Neonate* *

	% of Fractions			
	I	II	III	IV
Total lens extract	30.4	26.7	19.1	28.8

* Barbital buffer solution; pH 8.6; ionic power 0.1.

TABLE 7.—*Agar Microelectrophoresis of the Lens of a Neonate* *

	Relative Mobility of Fractions	% of Fractions
Rapid fraction	1.5; 1.2; 1.1	1.9
α-Crystallin	0.94	44.3
Middle fractions	0.67	2.2
	0.55	4.0
	0.47	2.6
	0.40	10.2
	0.30	3.45
Slow fractions	0.23	17.1
	0.11	13.3
	0.0	0.8

* Barbital buffer solution; pH 8.4; ionic strength 0.03; temperature 25 C; potential gradient of the electric field 10 V/cm. for 25 min.

fractionation of proteins in older lenses cannot be carried out to an extent sufficient to study and to interpret the changes which occur. High-tension electrophoresis, however, has made it possible to attain satisfactory separation of proteins, not only in older lenses but even in cataractous lenses. Determination of mobility is particularly important in order to identify and to compare the numerous protein fractions in various normal and particularly in opacified lenses.

By way of example we present a pherogram and a diagram of soluble lenticular proteins in a man aged 35 (Figs. 9 and 10). Considerable changes are immediately apparent, at this age, in the protein composition of the lens. The first pherogram was obtained at 4 C, with a potential gradient of the electric field 20 V/cm. for 25 minutes; the second was obtained at 4 C, with a potential gradient of the electric field 40 V/cm. for 22 minutes. We also present the pherogram and diagram of a cataractous lens, obtained under similar conditions (Figs. 9 and 10).

Considering the results obtained, it can be stated that as the technique is perfected and the power of separation increased the soluble lenticular proteins prove to consist of even more numerous fractions. The question may be raised as to when this increasing fractionation will stop. The progress made since the now classical conception of the three crystallins only hints at

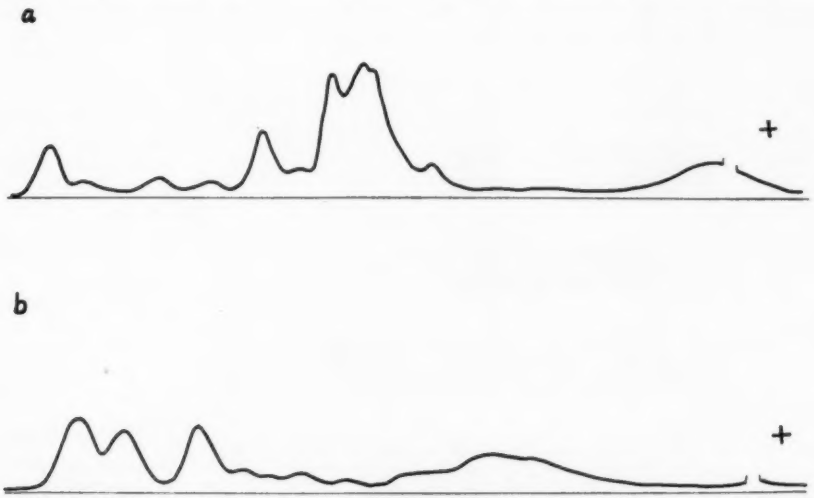

Fig. 7.—Diagrams of agar microphorograms of lens proteins in Scyllium canicula: temperature 4 C, potential gradient of electric field 40 V/cm. for 22 minutes. (rapid fractions not taken into account); (*a*) cortex and (*b*) nucleus.

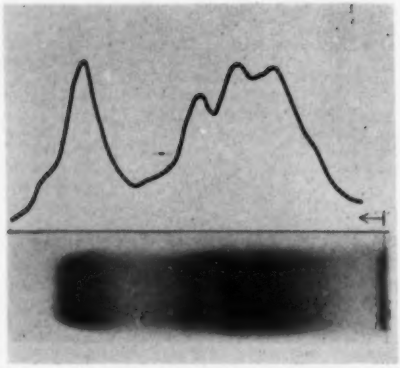

Fig. 8.—Paper pherogram and corresponding diagram of lens proteins of a neonate: total lens.

TABLE 8.—*Agar Microelectrophoresis at High Tension of the Lens of a Neonate* *

Relative Mobility of Fractions	% of Fractions
Rapid fractions 1.6; 1.3; 1.2; 1.1	3.4
α-Crystallin 0.95	37.9
0.65	2.2
0.58	1.4
0.54	3.0
0.45	2.7
0.40	10.1
0.32	4.3
0.25	6.8
0.20	14.2
0.13	4.0
0.10	9.2
0.02	0.7
—0.03	0.2

* Barbital buffer solution; pH 8.6; ionic strength 0.03; potential gradient of the electric field 20 V/cm. for 25 min.

the complexity of the protein composition of the lens.

It must be admitted, meanwhile, that the majority of the fractions obtained by high-tension electrophoresis are symmetrical and show no heterogeneity, although the middle fractions show certain peculiarities, such as the appearance of a fraction very widely stretched.

The question should also be raised as to the extent to which these numerous fractions correspond with actually different proteins. Numerous so-called pure proteins, such as hemoglobin and lactalbumin, would seem to be composed in actual fact of more than one fraction when submitted to technically flawless electrophoresis. In cattle, for example, lactalbumin consists of two electrophoretic fractions, the appearance of which depends on the breed examined (Aschaffenburg and Drewry, 1957).

The mobility of α-crystallin differs according to whether it is found in an extract of the cortex or of the nucleus of the lens in young animals (e. g., okapi) and according to the degree of polymerization of this protein (Rabaey, 1958). On the other hand, one must take into account the possibility of secession or interaction between various proteins.

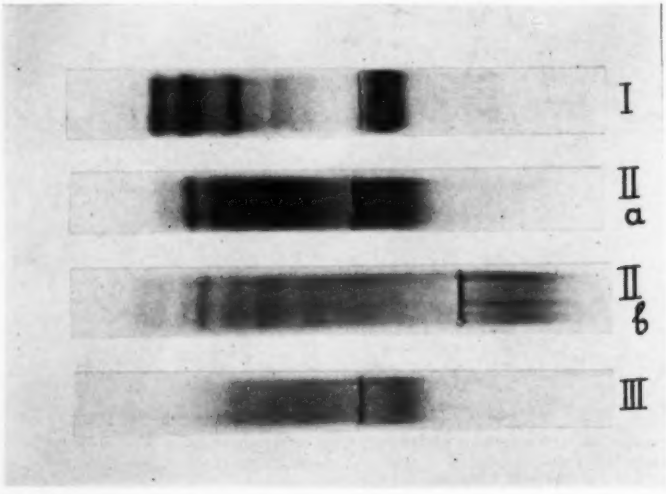

Fig. 9.—Agar micropherograms of proteins of the human lens. *I*, Neonate: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 min. *II*, (*a*) normal lens of a man, aged 35: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes; (*b*) lens of a man, aged 35: temperature 4 C, potential gradient of electric field 40 V/cm. for 22 minutes. *III*, Cataractous lens: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes.

However this may be, the separation of fractions which represent a modified condition of a given protein remains important, as it paves the way toward an investigation of the dynamics of lenticular proteins.

Summary

This is a report on the progress made by agar microelectrophoresis and high-tension microelectrophoresis as compared with the old techniques (free electrophoresis or paper

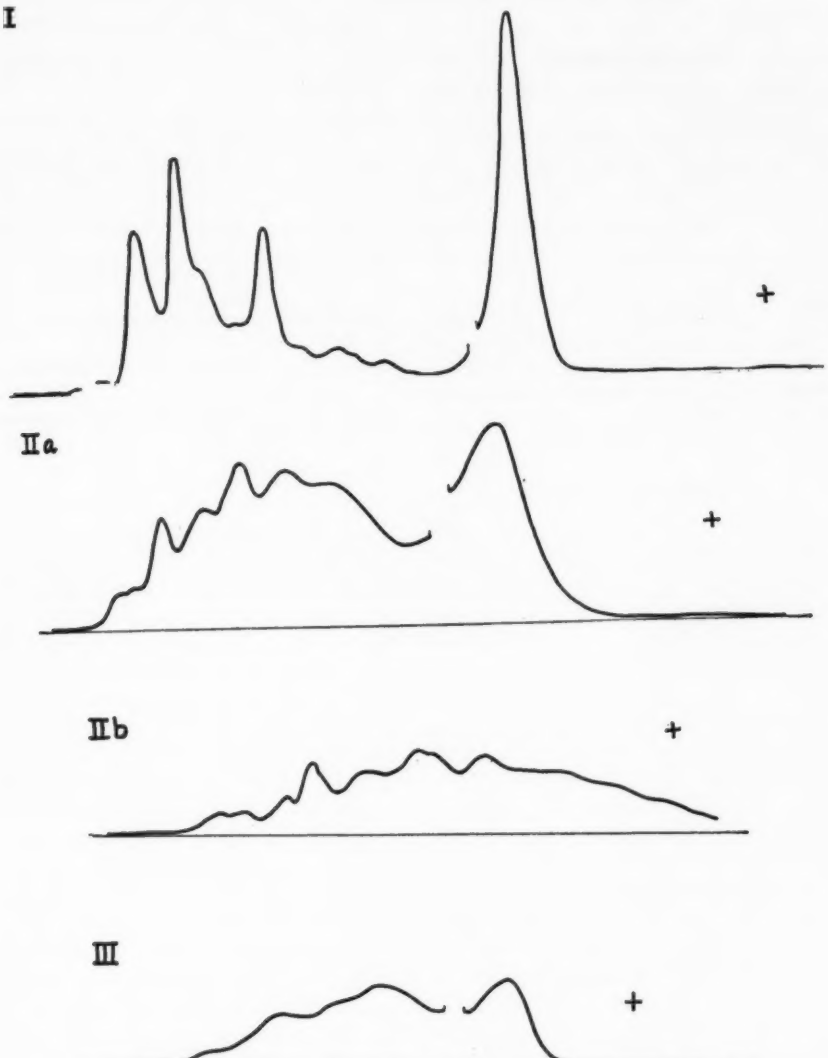

Fig. 10.—Diagrams of proteins of the human lens. *I*, Neonate: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes. *II*, (*a*) normal lens of a man, aged 35: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes; (*b*) normal lens of a man, aged 35: temperature 4 C, potential gradient of electric field 40 V/cm. for 22 minutes. The α-crystallin is not represented. *III*, Cataractous lens: temperature 4 C, potential gradient of electric field 20 V/cm. for 25 minutes.

electrophoresis). The new methods have considerably greater powers of separation. In infants, paper electrophoresis of lenticular proteins only reveals 4 fractions, whereas agar electrophoresis reveals 10 different fractions and high-tension microelectrophoresis reveals even 13 fractions.

2, Avenue Pasteur.

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Experimental Aberrant Lipogenesis

V. Biochemical Evidence of Oleate-Induced Lipid Formation in the Cornea

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The formation of a sudanophilic lipid by cells of the cornea when supplied with oleic acid or sodium oleate was first reported by Cogan and Kuwabara, in 1954.[1,2] Subsequent studies of this phenomenon, termed by them "aberrant lipogenesis," recently have been summarized by them in a series of papers analyzing observations on the serum,[3] substrate,[4] and tissue[5] factors involved. In these studies oleate-induced formation of lipid was demonstrated primarily by the accumulation of sudanophilic material within the cells.

Although Sudan staining has been widely used for the detection of lipids, it does not identify the type of lipid found in the corneal cells. It is conceivable that these cells, rather than synthesizing lipid, were concentrating the oleic acid as such in a manner similar to that which has been shown for amino acids.[6] Aware of this alternative explanation of their findings, Cogan and Kuwabara supported their contention that this is, in fact, lipogenesis by the corneal cells with evidence supplied by an additional staining technique, the Nile blue reaction.[2] The red staining of the lipid with Nile blue is customarily interpreted as indicating a neutral fat rather than a fatty acid.

However, definitive identification of the lipid must depend on biochemical methods, and it is obviously desirable to test these observations by means other than histochemical. Accordingly, experiments were undertaken to study by tracer techniques the phenomenon of oleate-induced lipid formation. The specific purpose of the present study was to determine whether this lipid was oleic acid or an oleate-containing lipid.

The evidence to be presented indicates that the lipid accumulated in corneal cells upon incubation with oleate is an oleate-containing lipid, presumably an ester, rather than concentrated oleic acid. The amount of oleate incorporated into this lipid by corneal cells of the rabbit can be correlated with the degree of sudanophilia of these cells.

Methods and Materials

For those experiments in which bovine corneal epithelium was used, sheets of the epithelium were removed by scalpel from the eyes of freshly slaughtered cattle at a local abattoir. The epithelium was collected in sterile tris (tris [hydroxymethyl] aminomethane) buffer, the collection flasks being weighed before and after the addition of the epithelium to obtain the wet weight of the tissue. At the laboratory, the epithelium was washed by immersion in two changes of tris buffer. Penicillin was added in a concentration of 500 units per milliliter to all media. Batches of epithelium, each usually consisting of 15 epithelial sheets and weighing between 500 and 1,000 mg., were then placed in sterile flasks. The medium, containing either tris buffer or horse serum, was added in a quantity of 2 ml. per epithelial sheet. Radioactive sodium oleate was added in a concentration of 7μM. per milliliter. The 1-C^{14} sodium oleate was prepared from 1-C^{14} oleic acid (Tracerlab) and diluted to the appropriate specific

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activity with nonradioactive sodium oleate prepared from oleic acid which had been purified according to the method of Schlenk and Holman.[7] The flasks were closed with rubber stoppers; the gas phase was air. The epithelium was incubated for 24 hours at 37 C.

For other experiments in which full-thickness rabbit corneal buttons were used, the corneas were excised from freshly killed albino rabbits and each cornea was divided into approximately 15 buttons. The buttons from two whole corneas were incubated for 48 hours at 37 C in horse serum to which radioactive sodium oleate, 7μM. per milliliter, had been added. Buttons from two whole corneas were similarly incubated in tris buffer containing 100 mg. % of glucose and radioactive sodium oleate.

After incubation of the bovine corneal epithelium or of the rabbit corneal buttons, the media were tested for bacterial contamination and tissue samples were fixed in 5% neutral formalin for subsequent histochemical study. The pH range of the media in all cases was between 7.0 and 8.0. $C^{14}O_2$ analyses after incubation indicated that an insignificant amount of the oleate had been oxidized under the conditions of the experiments.

The remainder of the corneal tissue was homogenized in a Potter-Elvehjem tissue grinder, and the lipids were extracted according to the method of Folch.[8] The petroleum ether extracts of lipids so obtained were passed through an anion-exchange-resin column, IRA-400 hydroxide form, to remove the unreacted oleate * carried over in the solvent. Aliquots of the effluent from the resin column were plated out to infinite thinness in stainless-steel planchets containing a disc of lens paper to give more even distribution of the solution. After evaporation of the solvent, the radioactivity was counted in a windowless flow counter.

To establish the efficiency of the anion-exchange resin in removing unreacted oleate present in lipid extract, several experiments were done. First, bovine corneal epithelium was incubated in tris buffer and oleate for a time (15 minutes) so short that the amount of oleate incorporated into lipid would be negligible; second, heat-inactivated epithelium was incubated in serum and oleate for 24 hours; third, epithelium was incubated for 24 hours in serum and oleate to which iodoacetate was added in a concentration sufficient to inhibit oleate incorporation completely. Untreated epithelium incubated for 24 hours in serum and oleate without inhibitors provided "normal control" values of the amount of oleate-induced lipid formation. After incubation, the epithelium was homogenized and the lipids were extracted. The radioactivity of the petroleum ether extracts before and after passage through the resin column was counted, and the data are presented in Table 1.

TABLE 1.—*Removal of Unreacted C^{14} Oleate from the Petroleum Ether Extract by the Anion-Exchange Resin*

Experiment	State of Tissue	Incubation Period	Inhibitor	Counts/Min. Recovered in Petroleum Ether Extract: Before Resin	Counts/Min. Recovered in Petroleum Ether Extract: After Resin
1	Untreated	15 min.	None	4,738 *	0 †
2	Heated at 56 C for 30 min.	24 hr.	None	6,720	0 †
3	Untreated	24 hr.	Iodoacetate (5×10^{-3}M)	4,785	0 †
Controls	Untreated	24 hr.	None	10,395	5,445

* Values represent the average of at least two observations except for Experiment 2.

† Negligible counts above background.

It is apparent from this Table that unreacted oleate is carried over in the petroleum ether extract but that the anion-exchange resin is highly efficient in removing it from the extract. The radioactivity remaining in the effluent from the resin

* Unreacted oleate refers to the sodium oleate or oleic acid which is not incorporated into lipid by corneal cells. It is believed that the oleate which is incorporated into lipid by corneal cells is esterified with one or more alcoholic compounds, such as glycerol or cholesterol.

TABLE 2.—*The C^{14} Oleate Incorporation by Bovine Corneal Epithelium in Tris Buffer and in Serum*

Experiment	Tris-Oleate *	Serum-Oleate *
1		6,600
2	1,500	9,200
3	4,200	8,300
4	2,900	7,200
5	5,400	7,100
6	3,100	6,300
7	4,800	8,600
Mean	3,650±1,270 (S. D.)	7,614±950 (S. D.)

* Counts/min/gm. epithelium (wet weight)/24 hr., corrected to an initial specific activity of 1000 counts/min/μM.

TABLE 3.—*The C^{14} Oleate Incorporation by Rabbit Corneal Cells in Tris Buffer and in Serum*

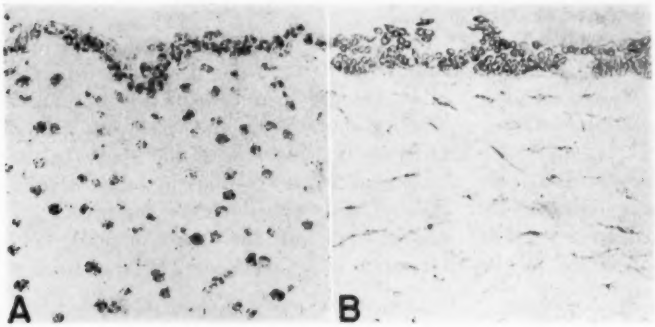
Tris Oleate *	Serum Oleate *	Sudanophilia of Stained Sections: Tris Oleate	Sudanophilia of Stained Sections: Serum Oleate
542	4,916	0	++++
141	3,587	0	++++
410	6,442	0	++++

* Counts/min/2 corneas/48 hr. Specific activity of oleate is equal to 2,600 counts/min/μM.

column, in the control experiments, represents that oleate which is incorporated, presumably by a process of esterification, into lipid.

Results

The amount of C^{14} oleate incorporated into lipid by bovine corneal epithelium incubated for 24 hours in serum and in tris buffer was determined. The reproducibility of the results, with use of the techniques described, is shown by the values recorded in Table 2. The range of the values obtained from the cells incubated in tris buffer (1,500 to 5,400 counts per minute per gram per 24 hours) was greater than that obtained from the cells incubated in serum (6,300 to 9,200 counts per minute per gram per 24 hours). The results indicated that consistently more oleate was incorporated in the presence of serum than of tris buffer. The average amount of the oleate incorporated in tris buffer was 3,650 counts per minute per gram per 24 hr., while in serum it was slightly more than twice this value (7,614). However, the dependency of the oleate-induced lipogenesis on the presence of serum in the bovine corneal epithelium was not as striking as that previously observed in rabbit cornea by histochemical techniques. Staining of the bovine corneal epithelium incubated in serum and oleate revealed that only sparse amounts of sudanophilic material were demonstrable. It appeared that removal of the epithelium by scraping the cornea had altered the capacity of the cells to form fat. Experiments were, therefore, conducted with use of rabbit cornea in place of the bovine corneal epithelium. To compensate for the smaller amount of tissue the incubation period was lengthened from 24 to 48 hours. The amount of oleate incorporated into lipid by full-thickness rabbit corneal buttons when incubated in serum and oleate and in tris buffer and oleate was determined and correlated with the sudanophilia of the stained sections. These data are presented in Table 3. The amount of oleate incorporation by cells incubated in serum is approximately 10 times that of



The comparison of the oleate-induced lipogenesis observed in serum (*A*) and tris buffer (*B*). The sections were stained with Sudan IV but not with hematoxylin.

cells incubated in tris buffer. The chemical findings were supported histochemically (Figure), in that the intracellular sudanophilic droplets were present in abundance in the epithelium, stromal cells, and endothelium of corneal buttons incubated in serum and oleate, whereas no sudanophilia was demonstrable in corneal buttons similarly incubated in tris buffer and oleate.

Recent observations by Cogan and Kuwabara indicate that palmitate and stearate are similarly incorporated by rabbit corneal cells into a lipid which is nonsudanophilic, but which is demonstrable histochemically as birefringent crystals.[9] Preliminary studies of the incorporation of radioactive palmitate as determined by the methods described here confirm their observations. Thus, in one experiment in which rabbit corneal buttons were incubated for 48 hours in tris buffer and palmitate and in serum and palmitate, the incorporation of 1-C^{14}-palmitate in tris buffer was 400 counts per minute per two corneas per 48 hours, while in serum it was 6,300 counts per minute per two corneas per 48 hours. The initial specific activity of palmitate in this experiment was 5,000 counts per minute per micromol.

Comment

The data presented demonstrate the in vitro incorporation of C^{14} oleate into lipid by both bovine and rabbit corneal cells. The lipid accumulated in the cells is a substance other than oleic acid. Since any unreacted sodium oleate or oleic acid would have been removed by the anion-exchange-resin column, the radioactive substance detected after the resin treatment is most likely some form of an oleate ester. Investigations are currently being conducted to establish this substance as a glyceride, a phosphatide, or an ester of cholesterol.†

As in the experiments reported in previous papers in this series on aberrant lipogenesis, serum enhanced to a marked degree the incorporation of oleate in the rabbit cornea. However, by the tracer technique a more quantitative evaluation of this phenomenon was possible. A tenfold increase was observed in the oleate incorporation by the rabbit corneal cells when serum replaced tris buffer as the incubating medium. The mechanism by which serum acts to increase markedly oleate-induced lipid formation is not known. It is possible that serum in some way facilitates entry of oleate into the cell; on the other hand, it may supply co-factors necessary for optimal lipid formation.

An important question concerning the experiments reported here is whether the oleate-induced lipid formation represents net synthesis rather than a process of exchange esterification. While the present study does not unequivocally demonstrate net synthesis, certain observations suggest that net synthesis occurs. First, oleate-induced lipid formation requires energy derived from anaerobic metabolism, as indicated by the complete inhibition of the process by iodoacetate (Table 1). Partial inhibition of the process by a nitrogen atmosphere ‡ indicates that energy derived from aerobic metabolism is also necessary. Second, the biochemical demonstration of the incorporation of oleate into lipid is associated with the concomitant accumulation of intracellular sudanophilic material by the corneal cells. Autoradiographic preparations further show that radioactive oleate is present within corneal cells after incubation in locations apparently identical with those of the sudanophilic droplets.

Summary

A method of investigation with use of a radioactive tracer is described whereby oleate-induced lipid formation was studied. The incorporation of C^{14} oleate into a lipid or lipids is demonstrated and supported by histochemical observations in rabbit corneal cells. The essential role of serum for the augmentation of the oleate-induced lipid formation is again noted. The lipid formed

† Ciccarelli, E. C.: Unpublished observations.

‡ Hill, K.: Unpublished observations.

is a substance other than free oleate and is presumably formed by esterification of the oleate.

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Keratoplasty for Fuchs' Dystrophy

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Until recently many ophthalmologists[1,3,7,8] considered that the prognosis of keratoplasty for Fuchs' dystrophy was poor. In 1952, however, Stocker[12] reported two successful grafts for Fuchs' endothelial and epithelial dystrophy. In 1953, Paton[4] included the results of keratoplasty in 13 cases of Fuchs' dystrophy, with five successful grafts. In 1956, Stocker[10] reported 12 more cases, with good results in seven eyes, fair results in two eyes, and failure in three eyes.

The present study reports the outcome of keratoplasty for Fuchs' dystrophy performed on 20 eyes of 18 patients at the Manhattan Eye, Ear, and Throat Hospital from 1952 to early 1957, which were followed from 3 to 36 months (average, 11.4 months). Five patients (six eyes) were from the cornea clinic of the hospital, and the others were private patients of Dr. Paton (eleven patients, twelve eyes), Dr. H. Katzin (one patient, one eye), and Dr. A. I. Turtz (one patient, one eye).

Twenty-three keratoplasties were performed in the 20 eyes. Twenty-two were partial penetrating grafts, including nineteen of 6 mm. and three of 7 mm. diameter. In one case, a 7 mm. lamellar graft was used. Two patients had bilateral keratoplasty, and three required reoperation because the original graft became cloudy. In two of the latter cases, corneal abscesses caused the clouding and the second graft remained clear; in the third case, the first graft clouded after cataract extraction and the second graft also clouded.

Results of Keratoplasty for Fuchs' Dystrophy

As is shown in Table 1, clear grafts were obtained in 14 (70%) of the total series of 20 eyes. Results in eyes with early or moderate disease, however, were strikingly better than results in eyes with advanced dystrophy. This observation agrees with earlier observations by Paton[4,5] and Stocker.[9,10]

TABLE 1.—*Results of Keratoplasty for Fuchs' Dystrophy in Twenty Eyes of Eighteen Patients*

Stage	Eyes, No.	Clear Grafts, No.	Cloudy Grafts, No.
Early to moderate	12	10 (83.4%)	2 (16.6%)
Advanced	8	4 (50.0%)	4 (50.0%)
Totals	20	14 (70.0%)	6 (30.0%)

In his earlier report, Stocker stated his belief that the chances of successful grafting for Fuchs' dystrophy were greater if the area of the dystrophy were surrounded by an area of healthy endothelium and if all, or nearly all, of the degenerated area was replaced by healthy donor tissue. He also recommended the use of donor material from persons under the age of 40 because of the higher incidence of endothelial changes in older eyes. Goar[2] reported that 6% of persons over 40 have central endothelial degenerative changes. In his later report, Stocker[10] divided his 12 cases into those with favorable and unfavorable prognosis. The cases classed as favorable comprised those with a circle of healthy corneal tissue surrounding the central dystrophy; those classed as unfavorable were the eyes with dystrophy involving the entire cornea. Of seven cases in the first, or favorable, group, six had clear grafts and the seventh was fairly satisfactory. In the second, or unfavorable, group, one had a clear graft; one a fairly clear graft, and three were failures.

In the present series, 12 eyes were classed as early or moderate dystrophy and 8 as advanced cases. The exact area of involve-

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TABLE 2.—*Clear Grafts Following Keratoplasty for Fuchs' Dystrophy—Earlier Study by Dr. R. T. Paton*

	Vision		
	Pre-operative	Post-operative	Follow-Up Mo.
5 clear grafts with visual improvement & no changes in lens	20/40	20/20	6
	20/70	20/60	3
	20/200	20/80	6
	20/400	20/25	9
	15/400	20/40	44
3 clear grafts followed by lens changes but no cataract extraction	20/400	20/300	8
	Hm.	20/60	30
	Hm.	20/70	35
5 clear grafts followed by lens changes & cataract extraction	Graft Cloudy 3		Graft Clear 2

ment was not recorded. The preoperative vision in the early or moderate cases were 20/400 in five cases, 20/200 in two cases, 20/100 and 20/70 in one case each, 20/40 in two cases, and 20/30 in one case. In the advanced cases, vision was no better than counting fingers at 3 ft. in any one instance. In the first group, 83.4% had clear grafts, and in the second, 50% (Table 1). Thus, the results of keratoplasty for Fuchs' dystrophy in this series are successful in a high proportion of early and moderate cases and considerably less successful in advanced cases.

Cataract Associated with Fuchs' Dystrophy

In evaluating final vision after keratoplasty for Fuchs' dystrophy, the frequency of associated cataract is an important consideration. The cataract is sometimes present at the time of grafting, but more often it appears later. This complication has also been observed by Stocker [12] and Paton.

Table 2 summarizes Paton's earlier experience with 13 cases. Five of these eyes had clearly improved vision with no cataracts. Three had decidedly improved vision over a considerable period of observation in spite of lens changes. In five instances successful keratoplasty was followed by sufficient lens opacity to warrant cataract extraction in the hope of improving vision. In three of these cases the graft became cloudy after the cataract operation, and in two they remained clear.

Stocker [11] has also had both successful and disappointing results in cases requiring both keratoplasty and lens extraction. Recently he has performed both operations at the same time when a well-developed cataract was present at the time of grafting. This method has also produced irregular results, but the series is still too small to permit conclusions.

In the present series, 12 of the 20 eyes had cataracts which interfered with vision. Four, including one case with bilateral cataract, have not yet been operated on. Table 3 summarizes the results of intracapsular extraction in seven eyes after keratoplasty.

In the four eyes with clear grafts at the time of cataract extraction, the operation was performed 3, 7, 11, and 12 months, respectively, after the corneal grafting. In the cases operated on 7 and 12 months after keratoplasty, the graft remained clear; in those operated on 3 and 11 months after grafting, the cornea became opaque. Of particular interest is one patient who had bilateral keratoplasty with clear grafts and bilateral cataract extraction. The cataract was extracted from the first eye three months after grafting, and the cornea later became opaque. When the second eye also developed cataract, operation was deferred until 12 months after keratoplasty, and the graft remained clear. While the number of cases is still small and the results somewhat irregular, it is at present the policy of the Manhattan Eye, Ear, and Throat

TABLE 3.—*Results of Cataract Extraction in Seven Eyes After Keratoplasty*

Graft at Time of Cataract Extraction	Eyes, No.	After Extraction	
		Graft Clear	Graft Cloudy
Opaque	3	0	3
Clear	4	2	2
Total	7	2	5

TABLE 4.—*Postoperative Vision Following Successful Keratoplasty for Fuchs' Dystrophy*

Vision	Eyes, No.
20/20 — 20/40	4 (28.7%)
20/40 — 20/80	6 (42.9%)
20/80 — 20/200	2 (14.2%)
20/200 or less	2 (14.2%)
Total	14 (100%)

Hospital to postpone cataract extraction in these cases until at least a year after karatoplasty.

Final Vision After Successful Keratoplasty for Fuchs' Dystrophy

Table 4 shows the vision in the 14 eyes with clear grafts and comprises both favorable and unfavorable cases. The tabulated eyes include two which have had second grafts because the first ones clouded, two with grafts which remained clear after cataract extraction following keratoplasty, and five eyes with clear corneas but with vision variably reduced by lens clouding. A total of 71.5% had final vision between 20/20 and 20/80, which represents a high level of improvement compared with the preoperative vision.

Comment

This study confirms the more recent view that keratoplasty offers a favorable prognosis in cases of Fuchs' dystrophy, especially in early and moderate cases. Fourteen of twenty eyes had clear grafts an average of 11.4 months after keratoplasty and a high level of visual improvement, in spite of cataracts in some cases. Particularly striking is the improvement noted in three of Paton's earlier patients with preoperative vision between hand movements and 15/400 who maintained vision of 20/40 to 20/70 from three to four years after operation (Table 2).

Partial penetrating grafts were used in all but one case, for which a 7 mm. lamellar graft was used. The choice was based on the nature of the disease, which is primarily endothelial. The purpose of the lamellar graft is to create a cicatrix between the host and donor tissue, leaving the anterior portion of the cornea clear, a procedure which seems unsuitable for Fuchs' endothelial dystrophy. In addition, the excellent results obtained in this series with penetrating grafts suggests that they are to be preferred.

Because only three grafts of 7 mm. diameter were used in this series, comparison of results using grafts of larger and smaller size is not possible. Two of the 7 mm. grafts were placed in early to moderately affected eyes and one, in an eye with advanced Fuchs' dystrophy. The first two were successful and the second was a failure, suggesting that the stage of the disease rather than the size of the graft may be the determining factor. In favor of using the larger graft is the fact that they replace more of the diseased tissue. On the other hand, larger grafts are more subject to complications.[4] In view of this consideration and the success achieved in all stages of the disease with 6 mm. grafts, the smaller graft seems indicated in most cases.

The high incidence of cataracts associated with Fuchs' dystrophy raises questions which cannot as yet be answered. The small number of cases in this and other reports does not warrant an assumption that cataract is a part of the picture of Fuchs' dystrophy. It seems more probable that corneal grafting, like operations for glaucoma, hastens the development of incipient lens changes. Whatever the relationship, the management of a patient with Fuchs' dystrophy and cataracts poses a difficult problem. While results are often discouraging, improvement—which may be striking—does occur in some of these cases.

The problem of cataract in an eye with Fuchs' dystrophy also raises the question of handling a patient with cataract and very early endothelial dystrophy in the form of cornea guttata. This combination is relatively frequent, and ophthalmologists have been concerned as to the possibility of the

cornea becoming opaque after cataract extraction in patients with this form of dystrophy. Some surgeons believe that a scleral incision should be used in order to avoid incision in the cornea and consequent access of aqueous to the superficial corneal tissue. However, the degenerated endothelium in this condition is central, and the process is entirely different from that of peripheral degeneration with Hassal-Henle bodies. Both Stocker [11] and Paton believe that the important consideration is avoidance of injury to the endothelium rather than the site of the incision. They therefore advocate the usual incision at the limbus.

Summary

The results of keratoplasty for Fuchs' dystrophy in 20 eyes of 18 patients are analyzed. The grafts remained clear in 14 cases, or 70% of the series. Grafts remained clear in a high proportion of cases of early and moderate Fuchs' dystrophy (83.4%) but in a smaller proportion of advanced cases (50%).

Associated cataract occurred in over one-half of the cases; results of cataract extraction after keratoplasty are irregular and are rather frequently followed by clouding of the graft.

Visual improvement is notable in eyes with clear grafts, sometimes despite lens clouding.

Penetrating grafts are recommended as logically more suitable to endothelial disease than lamellar grafts. Grafts 6 mm. in diameter give excellent results; in view of the added risk of complications following larger grafts, they are preferable in most cases.

The presence of cataract in these cases raises the question of cataract extraction in early cornea guttata. This is a distinct process, and the usual limbal incision is recommended.

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Keratoplasty for Keratoconus

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For some time ophthalmologists have recognized that keratoplasty corrects keratoconus in a large percentage of cases. There is, however, some difference of opinion as to the optimal size of the grafts. Castroviejo [1] and others have suggested a large graft which replaces the entire conus, because the high corneal curvature remaining after a smaller graft may cause a high enough degree of astigmatism and myopia to interfere seriously with vision. Paton [2] and others, however, have found that, while a larger graft may at times be desirable, in most instances the visual problems following a small graft are not severe enough to warrant risking the complications which are frequently associated with large grafts. The purpose of the present study is to evaluate the vision and refraction errors following 6 and 7 mm. corneal grafts for keratoconus.

Description of the Series

The records of keratoplasty performed in 109 eyes of 81 patients have been analyzed. Only cases in which the fate of the graft was known and the postoperative refraction was stabilized have been included in the study. The patients came from the private practice of Dr. Paton and Dr. H. Katzin and from the cornea clinic of the Manhattan Eye, Ear, and Throat Hospital. The majority were patients of Dr. Paton.

The operations were performed between July 1, 1953, and Aug. 1, 1957, and the average follow-up period was 16.4 months. Circular penetrating grafts were used in all cases. In 95 cases the graft was 6 mm. in diameter, and in 14 eyes a 7 mm. graft was used.

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TABLE 1.—*Results of 112 Keratoplasties for Keratoconus in 109 Eyes*

Graft Size, Mm.	Eyes, No.	Clear Grafts	Cloudy Grafts
6	95	90 (94.7%)	5 (5.3%)
7	14	13 (92.9%)	1 (7.1%)
Totals	109	103 (94.5%)	6 (5.8%)

Results of Keratoplasty for Keratoconus

Grafts remained clear in 103 (94.5%) of the 109 eyes operated on. Three eyes required a second operation because the original graft became cloudy, making a total of 112 keratoplasties in 109 eyes. In addition, synechotomies were necessary in four cases in order to preserve the clarity of the graft. Table 1 shows the clarity of the final graft in the entire series and in cases with 6 and 7 mm. grafts. Considering the small number of 7 mm. grafts, the differences in results cannot be considered statistically significant, but they are consistent with experience that smaller grafts are less subject to complications than larger grafts.

After operation 95 patients were made comfortable with ordinary glasses, while 8 preferred contact lenses. Table 2 shows the corrected postoperative vision of the 109 eyes. In most instances vision was excellent

TABLE 2.—*Corrected Vision in 103 Eyes with Clear Grafts After Keratoplasty for Keratoconus*

	Eyes, No.		
Corrected Vision	6 Mm. Graft	7 Mm. Graft	Total
20/20 — 20/30	67	10	77
20/40 — 20/50	18	1	19
20/60 — 20/70	4	1	5
20/80 — 20/100	1	1	2
Totals	90	13	103

TABLE 3.—*Correction of Astigmatism in Ninety-Nine Eyes Wearing Ordinary Glasses After Keratoplasty for Keratoconus*

Graft Size, Mm.	Eyes, No.	Av. Correction
6	86	—2.55 Comb. with 4.91×—
7	13	—2.85 Comb. with 5.21×—
Average for Total 99 Eyes		—2.58 Comb. with 4.95×—

and satisfactory to both the patient and the surgeon. Of the total series, 74.7% had 20/30 vision or better and 93.2% had 20/50 or better. While a slightly larger percentage of those with 7 mm. grafts had 20/30 or better, a considerably larger proportion of those with 6 mm. grafts had 20/50 or better. Considering the small number of 7 mm. grafts, comparison of the results is not practical, but it is evident that the 6 mm. grafts do achieve excellent vision.

The results of refraction were available for only 99 eyes; in the other cases vision was recorded only with contact lenses. Minus cylinders were used in all analyses. As is shown in Table 3, a relatively high degree of astigmatism was present in most cases. In one instance 20/25 vision was achieved without a cylinder, but cylinders were required in all other cases. The highest was 13 D., which gave the patient satisfactory 20/30 vision. In all cases the correction was satisfactory.

While theoretically a 7 mm. graft should result in less myopia and astigmatism than a 6 mm. graft, the opposite is the case in this series. All that can be conclusively stated, however, is that the 6 mm. grafts left only refractive errors which could be adequately corrected, usually by ordinary glasses or occasionally by contact lenses.

Comment

The high percentage of clear grafts, the excellent average of corrected vision, and the presence of myopia and astigmatism only in degrees which could be satisfactorily managed by means of glasses corroborate the usual view that keratoplasty is a successful treatment for keratoconus. The operation is indicated when satisfactory vision cannot be obtained by means of contact lenses, when the patient finds contact lenses irritating, or when the disease is progressing rapidly.

While the number of cases with 7 mm. grafts is too small to permit comparison of results, the differences in clarity, vision, and astigmatism between patients having 6 mm. and those having 7 mm. grafts is slight. The figures indicate clearly that the results of the smaller grafts are highly satisfactory. In view of the increased incidence of complications following larger grafts, Paton[3] has found that the 6 mm. graft seems definitely preferable in nearly all instances. The 7 mm. graft can, therefore, be safely reserved for unusual cases of corneal thinness or very large keratoconus which seem to warrant the extra risk involved in using the larger graft.

The postoperative refraction, like the clarity of the graft, depends largely on surgical technique. Even slight tilting or protrusion of the graft gives rise to high astigmatic and myopic errors. The danger can be minimized by special attention to the following points: 1. The trephine should be placed exactly at a right angle to the cornea in order to avoid tilting. 2. The graft should be placed in an area of reasonably thick cornea so that a good position can be maintained. 3. The graft should be firmly fixed to the host cornea.

Some authors, like Philps and Fincham[4] and Pittar,[5] consider that a corneal splint should be used to keep the graft in position. In this series, the first stitch of the crossed overlying sutures was run through and over Paton's plastic guard, which protects the graft from cutting by the thread and also acts to some extent as a splint. Supplementary edge-to-edge sutures were used only when they seemed necessary for extra security.

Summary

The results of keratoplasty performed for keratoconus in 109 eyes of 81 patients are analyzed. In 95 cases the grafts were 6 mm. in diameter, and in 14 cases they were 7

mm. in diameter. The graft remained clear in 103 cases, or 94.5% of the cases. The corrected vision was 20/30 or better in 77 eyes (74.7%) and 20/50 or better in 96 eyes (93.2%). The average correction after operation was −2.58 combined with 4.95 ×−.

In this series, myopia and astigmatism were slightly higher following 7 mm. than following 6 mm. grafts. In view of the smaller number of 7 mm. grafts, the only permissible conclusion is that the results of 6 mm. grafts are highly satisfactory. The high cylinders were extremely well tolerated by most patients. A few required contact lenses.

In view of the added complications following larger grafts and the excellent results following 6 mm. grafts, the smaller graft may be considered preferable in most cases.

Since publication of this article, we have been using 0000000000 virgin nonabsorbable surgical (silk) sutures, threaded on an 83-4 Greishaber needle. There are several advantages in this fine suture, the main one being that in keratoconus it is possible to place these sutures without entering the anterior chamber.

Removal of the sutures is best accomplished with the use of a binocular operating-room microscope, a broken razor blade, and a Chandler forceps.

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Tonometry—Effect of Tonometer Footplate Hole on Scale Reading

Further Studies

ROBERT MOSES, M.D., St. Louis

In 1958 Moses and Hahn [1] investigated the inconsistency of the low scale readings of the Schiøtz tonometer noted in the report of Kronfeld [2] and found to cause unintelligible results in tonography.[3] We found that at low scale readings (high pressures) the cornea bulges into the hole of the tonometer footplate, unduly elevating the plunger and exaggerating the estimate of intraocular pressure.

At the request of the Committee for Standardization of Tonometers, attempts have been made to quantitate this effect further.

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From the Department of Ophthalmology and the Oscar Johnson Institute, Washington University School of Medicine.

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The research relating to this study was also financed in part under a grant to Washington University School of Medicine made by the Alfred P. Sloan Foundation, Inc. The grant was made upon recommendation of the Council for Research in Glaucoma and Allied Diseases. Neither the Foundation nor the Council assume any responsibility for the published findings of this study.

Method

A tonometer with four interchangeable footplates was secured, the hole diameters being 3.3, 3.7, 4.0, and 4.5 mm., respectively.* All measurements were made on patients by me, with use of the technique previously described of obtaining a scale reading first with the footplate with the

* Supplied by J. Sklar Manufacturing Company.

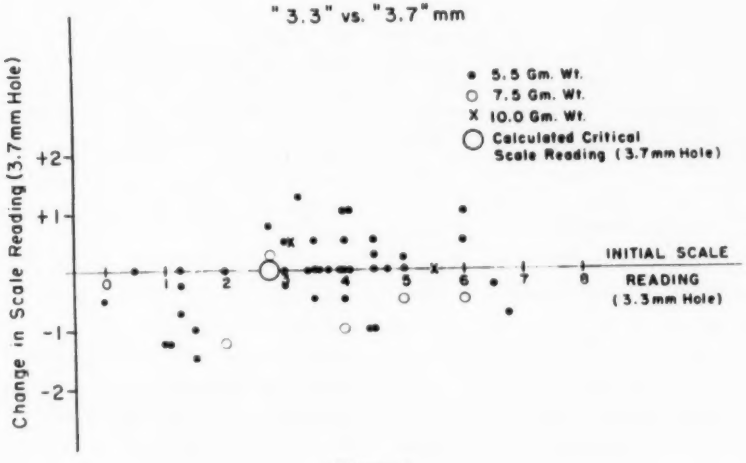

Figure 1

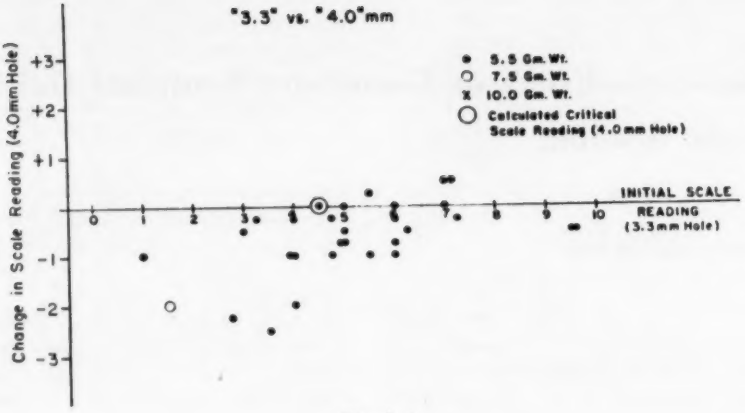

Figure 2

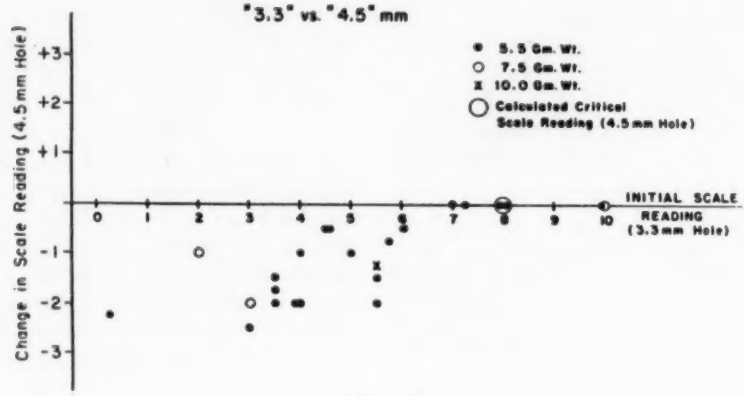

Figure 3

smallest hole (R_1) and then with a footplate with a larger hole (R_2). The results are presented graphically as the difference in scale reading ($\Delta R = R_2 - R_1$) vs. R_1 (Figs. 1 to 3).

Comment

Priestly Smith[4] pointed out that the area of cornea supporting the plunger is that in the relationship $P_t = \frac{W}{A}$, in which P_t is the intraocular pressure during tonometry (grams per square centimeter), W is the plunger weight (grams), and A is the area of the base of the indentation (square centimeters). The relation between P_t and scale reading (R) has been established experimentally.[5,6] The diameter of indentation (D_1) corresponding to A is readily calculated from $A = \pi \frac{(D_1)^2}{(2)}$. Thus the relationship between R and D_1 is easily plotted, the relationship being the same for any plunger load (Fig. 4).[7]

If D_1 is greater than the diameter of the footplate hole (D_h), the cornea adjacent to the indentation should rest against the footplate and the scale reading should be true. If D_1 is smaller than D_h, the cornea between the limit of the indentation and the edge of the hole may bulge into the gap and produce a spuriously low scale reading. The critical

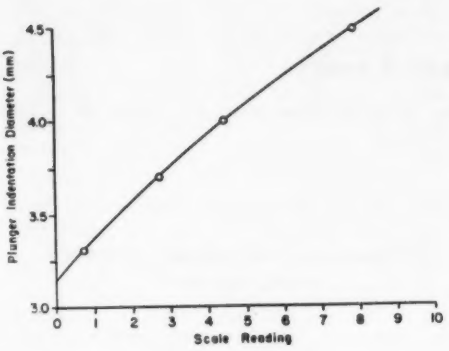

Figure 4

diameter $D_1 = D_h$ is indicated by large circles in the Figures. It is seen that the calculated critical diameter corresponds well with the experimental findings.

If our analysis is correct, the maximum footplate hole allowable for scale 0 would be 3.15 mm. diameter. The larger footplate holes were designed to retard the capillary rise of tears. Elimination of the gap between footplate and plunger might be expected to give difficulty due to tear fluid causing friction. However, we note (1) that an early Schiøtz tonometer we examined had a hole diameter of only 3.05 mm. and (2) that in the clinical tonometry involved in this study we encountered no difficulty with the 3.3 mm. hole.

It must further be noted that we used the 1955 calibration table for P_t[6] and the size of the footplate hole in the tonometer used by Friedenwald in developing these tables is unknown. Therefore, the tables themselves may be incorrect for small scale readings.

With respect to standardizing the hole size, there are two possibilities: (1) to make the hole no larger than 3.15 mm. diameter so that the tonometer may be consistent to scale 0 or (2) to adopt a larger standard hole and ignore readings in which the cornea may protrude into the hole, the pressure in such eyes being estimated by use of a plunger load heavy enough to produce a larger than critical scale reading for that hole.

Summary and Conclusions

The effect of the size of the hole in the footplate upon tonometer scale reading has been further investigated and has been found to accord well with theoretical considerations. Standardization of the hole size is necessary.

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Cytomegalic Inclusion Disease Uveitis

Report of a Case with Isolation from Aqueous Humor of the Virus in Tissue Culture

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Cytomegalic inclusion disease or salivary gland virus disease is a pathologic entity that has been known since 1904 by the large intranuclear and intracytoplasmic inclusion-bearing cells,[1] but it has recently been emphasized because the etiology has been clarified and its diagnosis has been facilitated by modern techniques. At first it was a pathologic rarity described only at autopsy and diagnosed as congenital syphilis[2] and amebiasis.[3] Later cases were described primarily as erythroblastosis fetalis,[4] keratomalacia,[5] whooping cough,[6,7] and toxoplasmosis.[8]

The salivary gland virus was originally described as large inclusion-bearing cells resembling those of cytomegalic inclusion disease found in the salivary glands[9] in a high percentage[10] of adult animals of numerous species.[11] It was found to be associated with a weak species-specific filtrable virus, transmissible to unborn or young animals for a few passages but not to adults,[12] presumably because of spontaneous asymptomatic infection. There were occasional exceptions to this benignity: a strain lethal for mice[13] or guinea pigs;[14] death at times in healthy animals used in debilitating experiments.[15]

The disease in humans is now better understood and correlates in many ways with its animal counterpart. Most commonly it is found at autopsy as asymptomatic inclusions in the salivary glands of children dying of any cause. Incidence of these salivary inclusions ranges from 12%[16] to 32%.[17] Generalized spread of the virus throughout the body may occur in 1.1% of children.[18] Adults seem to be susceptible to the virus dissemination throughout the body, particularly if affected by debilitating disease.[19] A number of cases have been described in pulmonary disease diagnosed as whooping cough or virus pneumonia.[6] However, the typical syndrome (Fig. 1) is of ophthalmologic interest because of possible ocular involvement. It is being recognized more frequently, since differential diagnosis is possible (Fig. 2), presumptive diagnosis can be assumed by periventricular calcification on

CYTOMEGALIC INCLUSION DISEASE

TYPICAL SYNDROME

1. Newborn or young infant, often premature
2. Fever
3. Jaundice, enlarged liver and spleen
4. Anemia, thrombocytopenia, petechiae, bleeding tendency
5. Cerebral calcification
6. Pneumonitis
7. Gastroenteritis

Figure 1

CYTOMEGALIC INCLUSION DISEASE

DIFFERENTIAL DIAGNOSIS

1. Syphilis	1. Serologic tests for syphilis
2. Tuberculosis	2. Skin tests, cultures
3. Erythroblastosis fetalis	3. Rh, ABO grouping, Coombs test
4. Sepsis	4. Bacterial cultures
5. Toxoplasmosis	5. Complement fixation and dye tests

Figure 2

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skull x-ray,[20] and definite diagnosis can be proven by the inclusion-laden cells in urine.[21]

Isolation of the virus in human tissue culture has been accomplished by Smith from salivary gland and kidney,[22] by Weller, Macauley, Craig, and Wirth from liver biopsy and urine,[23] and by Rowe, Hartley, Waterman, Turner, and Huebner from surgically removed adenoids.[24]

Formerly the disease was of little ophthalmologic interest, possibly because of insufficient examination, including examination under general anesthesia when possible. Keratomalacia without reported pathologic examination of the globes has been described,[5,16] and inflammation of the lacrimal glands without inclusion bodies has been reported.[5] The first case of proven ocular disease, reported from Lebanon,[25] in 1947, was in a one-month-old baby with necrotic membranous conjunctivitis which eroded the sclera and ciliary body. At autopsy inclusion bodies were seen in the tissues of the eye as well as in other organs. The case of Mercer, Luse, and Guyton [26] showed normal fundi on physical examination, but at autopsy focal retinal lesions 1×2 mm. were described. Lymphocytic and histiocytic infiltration and glial proliferation were present, but no organisms were seen. Another case was reported by McElfresh and Arey,[27] in which a round circumscribed patch of lighter color was seen in the choroid. No pathologic examination of the eye is mentioned. Mietke [28] described a case of dacryoadenitis and orbital swelling in a 59-year-old woman in which inclusion bodies were found.

The typical syndrome of cytomegalic inclusion disease was well described by Christensen, Beeman, and Allan [29] in a newborn infant with widespread visceral dissemination of the inclusion bodies. The eye showed a cataract, with retinal autolysis, hemorrhage, and gliosis. Inclusion-bearing cells were found in the retina and choroid.

Of the three cases reported by Weller et al.[23] in which virus was isolated, one showed chorioretinitis and optic atrophy and the ocular findings of another (E. Esp.) are to be discussed in this report.

Report of a Case

A full description of the general clinical features in this patient has been published.[30] The diagnosis has been proven by virus isolation from urine [23,30] and by finding of inclusion-bearing cells in urine and gastric washings.[31] Briefly, this was a full-term infant born May 9, 1956, weighing 3½ lb. While the infant was still in the premature nursery, at 5 weeks of age, an illness characterized by hepatosplenomegaly, periventricular cerebral calcification, and anemia developed. At 3 months of age, fever, vomiting, petechiae, thrombocytopenia, and leukocytosis appeared, followed by pneumonitis. This subsided, and by 6 months of age development was normal, except that hydrocephalus and mental retardation were apparent. At this writing (December, 1957) the child is alive but mentally retarded and hydrocephalic.

Ocular Examination.—The eyes were examined by pediatric residents under mydriasis as part of a screening routine for retrolental fibroplasia at 11 and 33 days of age and were considered normal. At 42 days of age, at the time of onset of illness, an area of chorioretinitis was described in the upper nasal quadrant of the right eye and the left eye was normal.

At 110 days of age, Aug. 27, 1956, the right cornea was clear. The anterior chamber showed 2+ flare but no cells. The pupils only dilated to 4 mm. A fundus drawing on Aug. 29, 1956 (Fig. 3), showed in the right eye a vitreous haze and an acute and a healed area of choroiditis superonasally with a perivascular exudate along a superior temporal vessel and a deposit below the macula. The left eye showed one healed pigmented lesion ½ D. D. in size in the nasal periphery (Fig. 4, inset). No fundus examination was done at that time under anesthesia, so it is possible that some of the lesions later seen were present and not found at the time of the first drawing. However, the progressive nature of the disease was confirmed by finding extension of

the superotemporal perivascular exudate, shown in Figure 3,* past the branching of the vessel one week later.

Subsequently the ocular condition became progressively worse throughout September. Examination at approximately weekly intervals showed progression of the uveitis to such an extent that it appeared likely the right eye might be lost. The paracenteses performed in September and October did not appear to slow or hasten the progress of disease. At the time of the next drawing, on Oct. 6, 1956, at 150 days of age, the right cornea appeared cloudy, the iris was vascularized, there was 2+ aqueous flare without cells in the anterior chamber, and the vitreous was very hazy. The superonasal lesion was larger, and additional lesions were seen inferonasally and in the temporal periphery (Fig. 4). The left eye was unchanged.

Gradually the ocular inflammation subsided. At 194 days of age, Nov. 19, 1956, the left eye exhibited a clear vitreous and four pigmented areas of healed chorioretinitis, of which only the nasal one had been previously seen (Fig. 5). The right eye showed a clear anterior chamber and less cloudiness of the media. The superonasal and inferonasal lesions appeared smaller, circumscribed, and healed. Bands protruded into the vitreous from the superonasal lesion. There was some atrophy and moderate over-all pigmentation of the healed areas but no sharply demarcated punched-out appearance. The optic nerves appeared white and atrophic. There was searching nystagmus.

The right eye continued to show clearing of the vitreous until January, 1957, when a recurrence of the vitreous haze and recrudescence of the temporal foci of choroiditis recurred. Some activity was evident in a drawing made at 9 months of age, on Feb. 5, 1957 (Fig. 6). The left eye still showed four healed areas. This appearance was confirmed at the time of ventriculography, Feb. 8, 1957, when the first fundus examination under general anesthesia was done.

By March 28, 1957, the vitreous had cleared in the right eye. On June 4, 1957, the eyes were infraverted and there was roving nystagmus and exotropia. The pigmented areas were shrinking and blanching. There was a flinch reaction to light.

Another recrudescence of inflammation in the right eye was noted July 17, 1957. There was increase in size and of the soft blurred grayish appearance of the foci of chorioretinitis in the right eye at 11 and 2 o'clock, with an increase in vitreous haze. The left eye did not change. This exacerbation subsided, and no recurrence of activity has been noted since. A final fundus drawing under general anesthesia in December, 1957 (Fig. 7), showed similar optic atrophy and an atrophic pebbled appearance in the retinas of both eyes. There was no vitreous haze in the left eye, and the previously described chorioretinal lesions were unchanged. The right eye showed flattening, shrinkage, and a diffuse slate-gray appearance of the previously described areas of chorioretinitis. No new lesions were seen, and no activity was noted.

Diagnostic Procedures.—An anterior chamber paracentesis was performed Sept. 18, 1956. Aqueous, 0.1 ml., was aspirated into a saline-moistened syringe. The specimen was contaminated with conjunctival fluid. One-half was shipped in melting ice to Dr. Thomas H. Weller, at the Harvard School of Public Health and was negative for virus growth.[30] The other half was placed on egg-albumen-coated slides and examined by the Papanicolaou technique. One cell with a large pale-staining nucleus, without chromatin, was found which was considered compatible with but not diagnostic of cytomegalic inclusion disease. The eye remained white before and after the paracentesis.

A second paracentesis was performed Oct. 19, 1956. Aqueous, 0.15 ml., was aspirated into a syringe moistened with Earle's basic salt solution and immediately deep-frozen for later tissue culture. Again there was no unusual reaction to the procedure.

* The figures 3 through 7, 11, and 14 through 20 may be found in the color plate.

A third paracentesis, with withdrawal of 0.15 ml. of aqueous, was done Oct. 26, 1956. The specimen was centrifuged, and the sediment was examined for inclusion-bearing cells. None were found. The supernate was shipped without refrigeration to Dr. G. Richard O'Connor, National Institutes of Health, Bethesda, Md. No anti-Toxoplasma antibodies were found by the agar-diffusion technique,[32] and no virus growth was obtained in tissue culture by Dr. Wallace Rowe, of the National Institutes of Health.[33]

A conjunctival smear for inclusion-bearing cells was suggestive but not diagnostic when examined by the Papanicolaou technique.

Laboratory Investigation.—The MAF line of embryonic fibroblasts * was grown in roller tubes, with use of 1 ml. of Eagle's basic medium[34] with 10% horse serum inactivated by heating to 56 C for 30 minutes. Antibiotics were added to a concentration of 0.1 mg. of penicillin, 0.05 mg. of streptomycin, and 50 units of nystatin (Mycostatin) per milliliter. Phenol red was used as a pH indicator.

* Obtained from Microbiological Associates, Inc., Bethesda, Md.

Fig. 8. — Fibroblasts stained with Giemsa, in situ in roller tube, low power. Scattered cells with enlargement of cytoplasm and early nuclear inclusions.

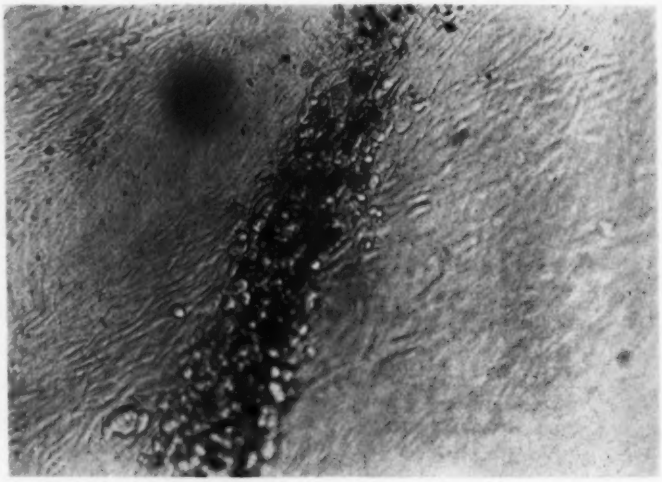

Fig. 9. — Low-power photomicrograph of unstained tissue culture in situ in test tube. Fifth passage of inclusion-producing agent, inoculum 0.2 ml. of supernatant fluid centrifuged free of cells. Photographed 30 days after inoculation, showing elongated area of enlarged cells with pigment-granule deposition in center.

Tissue-culture fluids were changed as necessitated by pH changes, usually every two to five days. For gross examinations, the roller tubes were examined unstained or the cells were stained in the tube with Giemsa stain. For histologic preparations, either the Cheatham technique[35] or preparations grown on coverslips in Leighton tubes were used. The fixative used was half ether and half 95% alcohol; stains were tetrachrome or hematoxylin and eosin with citric acid.

The aqueous specimen removed Oct. 19, 1956, and stored in the deepfreeze at −20 C was thawed March 9, 1957, and diluted 1:2 with Earle's basic salt solution. Four roller tubes of embryonic fibroblasts were inoculated with approximately 0.1 ml. aqueous dilution each. Fourteen days later granular areas were seen in two of the four tubes. Cells were removed by scraping with a platinum wire loop and inoculating a cell suspension and was inoculated into fresh roller tubes on March 26, 1957. After eight days, diffuse scattered enlargement and granularity of cells were seen (Fig. 8) and inclusion bodies were found in these cells. Supernatant fluid from medium changes was preserved in deepfreeze, as was one tube, when most cells were necrotic. At this point fresh cells for subculture became unavailable, and it was several months before a workable line of embryonic fibroblasts was obtained from the previous source.

It was then attempted to regrow inclusion-producing agents in a fresh line of the same cells. Supernatant fluid from medium changes and Seitz filtrates produced no growth. One tube which had been preserved with necrotic cells in situ gave positive results. Cytopathogenic changes occurred in two tubes inoculated with 0.2 ml. of cell suspension after 51 days, and a cell suspension was repassaged to fresh tubes eight days later. From this fourth subculture a strain of virus was obtained which produced cytopathogenic changes regularly in six to seven days when an 0.1 ml. inoculum of cell-free suspension (supernate centrifuged five minutes at 2,000 rpm) was passaged to roller tubes bearing 0.9 ml. of Eagle's medium (Fig. 9). Uninoculated control tubes were maintained at all times, except during a short period after the first subculture had been passed. Six passages have now been done. No similar cytopathogenic changes were seen in controls.

Evidence of antibody in the patient's serum to the cytopathogenic agent was obtained by neutralization tests, with use of a virus inoculum of 0.1 ml. of fresh cell-free suspension per total volume of 1.0 ml. in roller tubes. Serum specimens drawn September, 1956, and October, 1957, were

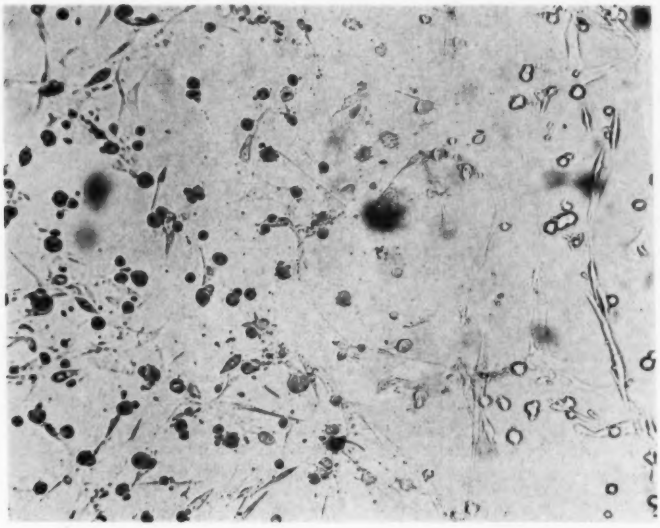

Fig. 10. — Low-power photomicrograph of unstained tissue culture in situ in test tube. Inoculum 0.2 ml. of fresh urine, 18 hours after inoculation. Most of the cells are shrunken, rounded, and translucent.

diluted logarithmically with culture medium, mixed in 0.1 ml. amounts with 0.1 ml. virus inoculum, and incubated at 37 C for one hour. Control tubes without serum showed earliest cytopathogenic changes in six to seven days. Serum dilutions were run in duplicate, and evidence of inhibition was considered to be failure to develop cytopathogenic changes in 30 days in both tubes. By this criterion, the serum of September, 1956, showed virus neutralization in 1:10 dilution and the serum of October, 1957, in 1:100 dilution. Since this was only one dilution different and the serum specimen of September, 1956, was slightly hemolyzed, it is not felt that this represents a rise in titer.

It was notable that the patient's urine contained very high quantities of the virus. Rapid cytopathogenic changes including the majority of cells in the tissue culture occurred in dilutions of 10^{-3} to 10^{-4} when urine was inoculated after 4, 5, and 16 months of disease (Figs. 10 and 11). Intranuclear inclusions were not developed in many cases, since the cells died too rapidly, although cytoplasmic changes were seen. However, by 18 to 20 months of age, urinary sediment had decreased, inclusion-bearing cells were not found in the urine, and inoculation of urine in 0.1 ml. amounts produced no virus growth or a sporadic positive plaque.

The observed microscopic effects without staining were as follows: with a virus inoculum of low titer, a few scattered areas would appear in 15 to 60 days, round to oval in shape, with the cells appearing round or oval, granular, translucent, and moderately enlarged (Fig. 12). Some of the cells appeared to drop out, and necrosis was evident in the center of the plaque. In some foci dark-brown granular pigmented areas appeared.

In preparations of higher potency the first lesions were of rather diffuse changes, with single cells becoming involved in 7 to 10 days. After this, generalized necrosis of the cells tended to appear rapidly.

If the highest titer inoculations of urine (0.2 to 0.3 ml. in 1.0 ml. of medium) were used, generalized rounding up of cells with a translucent appearance developed in one day (Fig. 10) and most cells were dead by three days (Fig. 13). Granularity of individual cells was minimal, and no pigmentation appeared. It is possible that this appearance may be a toxic, rather than viral, effect, since cytoplasmic changes appeared but definite intranuclear inclusions could not definitely be said to occur (Fig. 14).

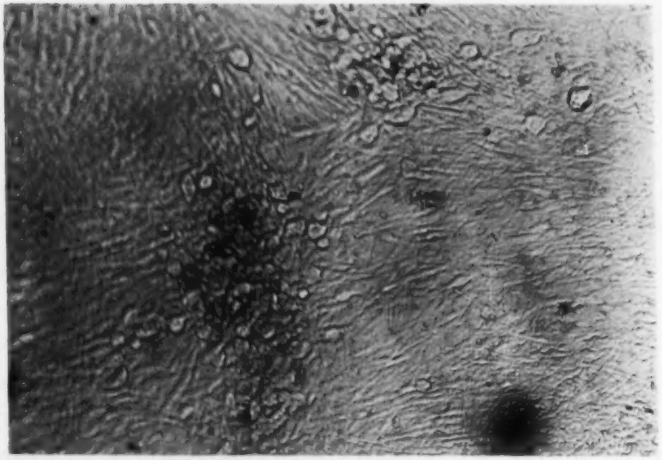

Fig. 12. — Low-power photomicrograph of unstained tissue culture in situ in test tube, sixth passage of virus. Two areas of typical round-to-oval shape showing spread of involved cells centrifugally and early pigment deposition in the larger.

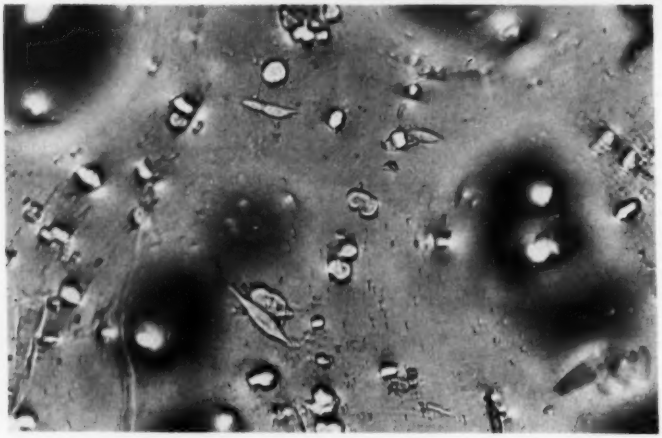

Fig. 13. — Low-power photomicrograph of unstained tissue culture in situ in test tube. Inoculum 0.2 ml. of fresh urine three days after inoculation. Generalized rounding up and transparent appearance of cells.

Typical viral effect in plaques showed the following histologic appearance, comparable to previous descriptions.[22-24] The affected areas were round to elongated (Fig. 15), and satellite plaques appeared after a time (Fig. 16). Centrifugal spread occurred, with necrosis of the inner cells in the area. The cells were readily identified when well involved by the altered size and dense cytoplasmic and dense nuclear stain.

Individual nuclear changes in the earliest affected cells at the periphery of a lesion were the appearance of one to several small nuclear granules (Fig. 17), which later clumped with or without the nucleoli, while the chromatin network became destroyed, to form one or more nuclear inclusions which might stain eosinophilic or basophilic. When fully developed, the intranuclear inclusion appeared granular with a darker center and lighter periphery, surrounded by a clear halo and nuclear membrane with clumped chromatin (Fig. 18). At times the center of the inclusion appeared to be one or more separate bodies (Fig. 19).

The cytoplasm might show either small granular inclusions of varying size arranged in a cap toward one end of the cell (Fig. 19) or a juxtanuclear eosinophilic area with fine granulation (Fig. 14) or both (Fig. 20).

The effect of storage on the virus was notable. None of the cell-free supernates stored at refrigerator or deepfreeze temperature for five months contained viable virus.

Figure 3

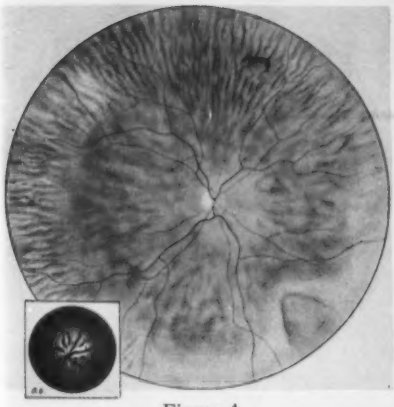

Figure 4

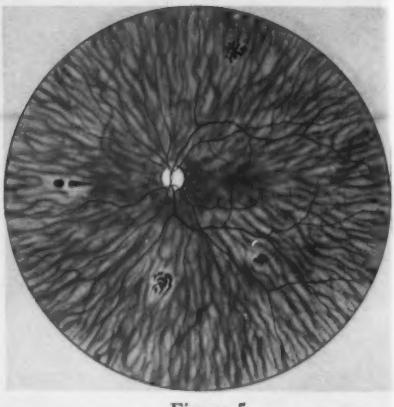

Figure 5

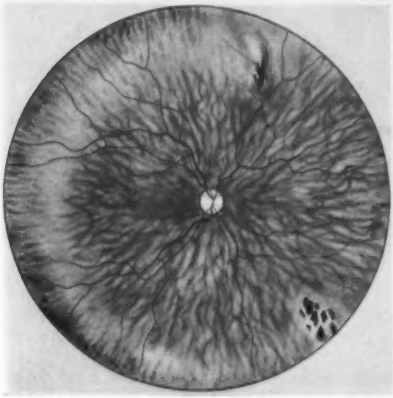

Figure 6

Figure 7

Fig. 3.—Right eye at age 112 days. Areas of acute and healing choroiditis, perivascular and macular exudates. (Reprinted by permission from the **New England Journal of Medicine** 257:805 Oct. 24, 1957.)

Fig. 4.—Right eye at age 150 days. Progression of uveitis with cloudy media, entire periphery diseased with areas at 11, 1, and 5 o'clock most markedly involved.
Inset: One healed area of chorioretinitis in nasal periphery, left eye.

Fig. 5.—Left eye at age 194 days. Vitreous clear. Four small granular non-destructive areas of chorioretinitis in periphery. Optic disc white and sharply demarcated.

Fig. 6.—Right eye at age 272 days. Moderate vitreous haze, especially temporally. Bands protrude into vitreous from superonasal shrinking lesion. Inferonasal lesion becoming smaller, with coarse pigmentary mottling. Optic nerve atrophy marked.

Fig. 7.—Right eye at age 1½ years. Vitreous clear. Entire retinal background appears moth-eaten and atrophic. Previous areas of chorioretinitis shrunken, discrete, and pigmented.

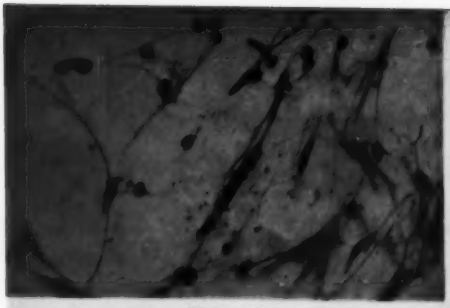

Figure 11

Figure 14

Figure 15

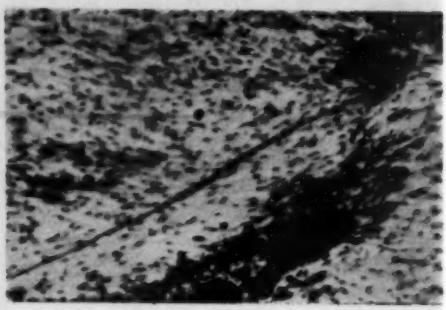

Figure 16

Figure 17

Figure 18

Figure 19

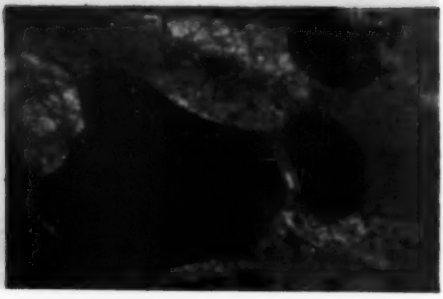

Figure 20

Urine specimens originally diffusely positive in five days in a dilution of 10^{-3} to 10^{-4} after one year of deepfreeze storage showed a few slowly developing cytopathogenic changes of a focal, rather than diffuse, nature in 10^{-1} dilution after 10 days.

The fact that a virus was isolated from the second paracentesis and not from the first or third warrants examination. There is a possibility that secondary aqueous was obtained, but no exacerbation of inflammation was ever noted after the paracenteses, and one month elapsed between the first and second. The third paracentesis specimen was centrifuged to remove cells before being sent without refrigeration for tissue-culture study, and no cells were found. Preservation of the second specimen was more careful and freezing more prompt than in the other two specimens.

No cells were seen at any time in the anterior chamber of the infant on slit-lamp examination, although flare was present. However, examination was always very unsatisfactory, and cells may have been present. Another possibility is that the aqueous culture tubes were contaminated by virus from urine culture tubes. Against this fact is the lack of development of any inclusions in any control tubes, as well as the different type of cytopathogenic change produced by more potent urine inocula.

Comment

It is felt that simultaneous occurrence of ocular and systemic disease is suggestive but not definitive evidence that the two diseases are of the same etiologic origin. The systemic disease is well proven in this instance by the clinical syndrome fortified by the x-ray demonstration of periventricular calcification, the pathologic evidence of inclusion-bearing cells in urine and gastric contents, and the culture of virus from urine in human tissue culture. To prove the etiology of this ocular disease, additional data other than temporal coexistence were obtained by isolation of the virus from aqueous humor after failure to demonstrate inclusion-bearing cells in the aqueous.

If this disease is as widespread as evidence may indicate,[16,24] it may possibly be a commoner cause of uveitis than previously suspected, since proven cases may show little histologic proof of inclusion bodies at autopsy[36] after the disease has apparently run its course. It is noteworthy that the two eyes in which histologic proof of inclusion bodies were found[25,29] were obtained at death during the acute illness. Conceivably eyes enucleated after a period of chronic inflammation might have lost the inclusions present in an acute stage.

Therefore, it is suggested that in cases of acute uveitis in children a pathologic ex-

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Fig. 11.—Coverslip preparation of tissue culture. Inoculum 0.2 ml. of fresh urine, 18 hours after inoculation. Generalized shrinkage and alteration of nuclear and cytoplasmic staining in majority of the cells. Tetrachrome; × 50.

Fig. 14.—Coverslip preparation of tissue culture. Inoculum 0.2 ml. of fresh urine, 60 hours after inoculation. Areas of pinkish-staining granular cytoplasm and clumping of nuclear material. Tetrachrome; × 215.

Fig. 15.—Coverslip preparation of tissue culture. Sixth tissue-culture passage of virus isolated from aqueous, 20 days after inoculation with 0.05 ml. of supernatant cell-free fluid. Discrete focus of cells with surrounding normal fibroblasts. Hematoxylin and eosin; × 50.

Fig. 16.—One end of same focus illustrated in Figure 9, showing central necrosis and pigment in larger area, and two daughter plaques. Hematoxylin and eosin.

Fig. 17.—Coverslip preparation of tissue culture. Fifth tissue-culture passage. Upper cells normal, well-developed eosinophilic and basophilic nuclear inclusions in center and lower cells beginning to show early changes. Hematoxylin and eosin; × 110.

Fig. 18.—Oil immersion. Fifth tissue-culture passage. Granular nuclear inclusion with dark inner core and lighter border, with chromatin clumping along nuclear membrane. Basophilic and amphophilic cytoplasmic granulation. Hematoxylin and eosin.

Fig. 19.—Cheatham preparation, oil immersion. Fifth tissue-culture passage. Central cell enlarged, with dense intranuclear inclusion showing smaller central bodies. Thin halo between inclusion and nuclear membrane. Cytoplasmic granules of varying size. Tetrachrome.

Fig. 20.—Cheatham preparation, oil immersion. Fifth tissue-culture passage. Eosinophilic paranuclear area in cytoplasm with separate granules in cytoplasmic periphery. Tetrachrome.

amination of the urinary sediment for inclusion-bearing cells be performed. At the present stage of our knowledge it would not be of help to demonstrate complement-fixing or virus neutralization antibodies in serum if a large proportion of the population shows similar findings.[24] A skull x-ray showing periventricular calcification would be strongly suggestive.

It has been attempted to determine ocular factors that would be of clinical value in aiding differential diagnosis. Rubella chorioretinitis would be suggested by the maternal history and the presence of cardiac lesions, cataracts, and hearing defects. Toxoplasmosis would present the greatest difficulty, as a syndrome of chorioretinitis with cerebral calcification, hepatosplenomegaly, and anemia was previously considered typical of toxoplasmosis. However, presumptive and definitive diagnosis is now possible in both toxoplasmosis and cytomegalic inclusion disease, although both diseases may coexist.[37] From an ocular standpoint, if the typical chorioretinitis of toxoplasmosis is a single or few macular or posterior polar lesions, with involvement of retina, choroid, and sclera, healing to leave punched-out areas of bared white sclera with surrounding pigment, then it may be contrasted with the pathologic picture seen in this case where the lesions were multiple and peripheral and showed less tissue destruction. In the case of Christensen et al.[29] the peripheral location and relatively mild involvement would tend to confirm this supposition. Only future proven cases will provide a definite answer.

Summary

A case of proven generalized cytomegalic inclusion disease is described. Chronology of ocular disease through chorioretinitis and optic atrophy is detailed.

An agent was isolated from the anterior chamber which produced inclusions in tissue culture similar to those previously isolated from the urine. It is felt that this evidence tends to establish the viral etiology of the ocular disease.

It is suggested that cytomegalic inclusion disease be considered as a cause of uveitis in children. Examination of urine for inclusions and skull x-rays are advocated for aid in differential diagnosis.

Cytomegalic inclusion disease should be considered with toxoplasmosis in the differential diagnosis of uveitis associated with cerebral calcification.

Dr. Rustin McIntosh, Professor of Pediatrics, Columbia University College of Physicians and Surgeons, gave permission to examine the patient. Tissue culture studies were performed in the laboratory of Dr. Calderon Howe, Department of Microbiology, Columbia University College of Physicians and Surgeons.

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The Nonelastic Nature of Pingueculas

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A pinguecula is a yellowish-white fluorescent mass situated over the sclera on one or both sides of the cornea. Its pathogenesis is far from clear, but it appears to be determined genetically and to assume tumorous proportions only in middle life or later.

Histologically pingueculas vary from masses of amorphous hyaline substance to masses of coiled and fragmented tissue that simulate pathologic elastic tissue elsewhere (e.g., pseudoxanthoma elasticum [1]), but they are unlike any tissue occurring normally about the eye. This amorphous and fragmented tissue stains regularly with orcein and resorcinol fuchsin dyes or by Verhoeff's method, whence it is called (degenerated) elastic tissue. It does not stain with Sudan and is variably colored by basic and acidic stains.

In 1949 Balo and Banga [2] described a nonproteolytic enzyme derived from pancreas which had the specific property of solubilizing elastic fibers. This enzyme, which is active on fresh or formalized tissue, was called by them elastase. It has come to be accepted as the most crucial test for true elastic tissue and has been employed particularly for differentiating fibers of connective tissue origin from those of elastic tissue origin. Elastase has no effect on components other than that of true elastic tissue or keratohyaline granules.[3] It is frequently used in demonstrating the specific dissolution of elastic tissue in the aorta and ligamentum nuchae.[4,5] It has also been used to show the true elastic nature of pseudoxanthoma elasticum, senile elastosis, and colloid millium.[1,5,6] To our knowledge, it has not previously been applied to pingueculas.

We applied the standard technique for enzymatic elastolysis to five typical cases of pinguecula. This was performed on fresh tissue and on tissue fixed in formalin. The procedure consisted of incubating the tissue in a solution of elastase * (10 mg/100 ml.)

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Howe Laboratory of Ophthalmology, Harvard University Medical School, and the Massachusetts Eye and Ear Infirmary. Public Health Service Fellow (Dr. Howard).

* Obtained from Worthington Biochemical Corporation.

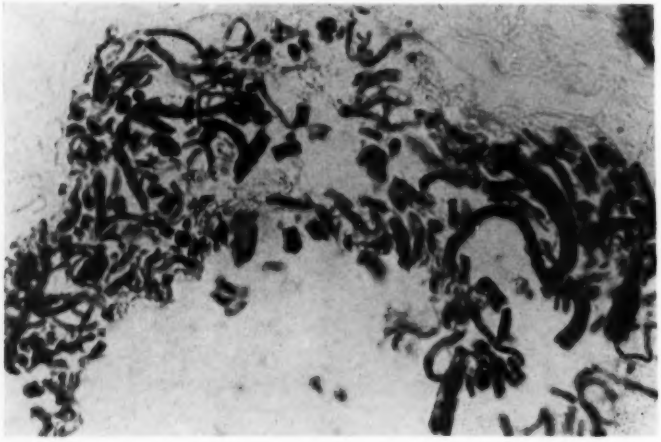

Fig. 1.—Pinguecula incubated for 24 hours in elastase and stained by Verhoeff's method. No elastolysis occurred with incubation.

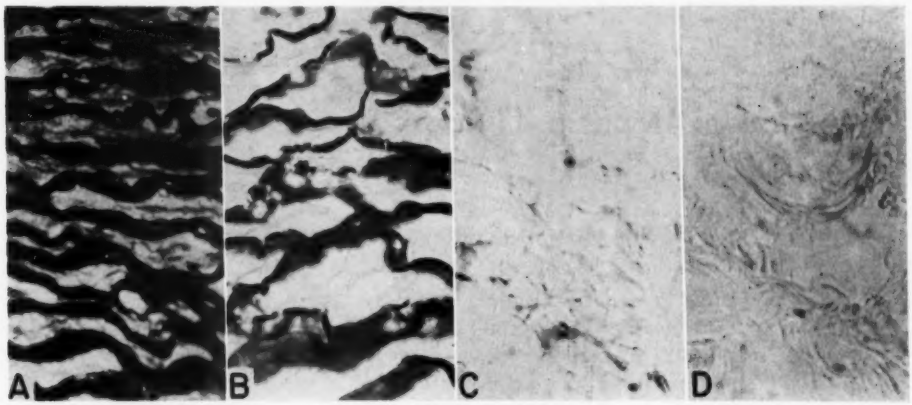

Fig. 2.—Strips of aorta stained by Verhoeff's method: *A*, without incubation; *B*, after 3 hours' incubation with elastase; *C*, after 6 hours' incubation with elastase, and *D*, after 24 hours' incubation with elastase. In contrast to pinguecular, the elastic aorta tissue shows a progressive elastolysis.

at 37 C in carbonate buffer (pH 8.8) for varying lengths of time, embedding the tissue in gelatin or paraffin, sectioning at approximately 10μ, and staining with Weigert's or Verhoeff's elastic tissue stain. The sections were compared with those of aorta tissue incubated for the same length of time under identical conditions.

The results may be summarized by stating that no effect was induced in the pingueculas even after incubation for as long as 24 hours (Fig. 1), whereas the elastic fibers of aorta tissue began to disappear in 3 hours and were completely abolished in 24 hours (Fig. 2, *A-D*).

Whether or not this disproves the elastic nature of pingueculas depends on one's criteria for definition of elastic tissue. If the current consensus is correct, that elastase is the one specific test for true elastic tissue (as shown for normal elastic tissue and certain elastic dystrophies of the skin), then pingueculas would have to be excluded from this category. Pingueculas are, according to this criterion, a pseudoelastic tissue.

243 Charles St. (14).

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Evaluation of Heparin Therapy of Senile Macular Degeneration

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Heparin therapy has been recommended for senile macular degeneration, in the belief that this treatment may improve the nutrition of the degenerating region. A recent report [1] states that more than half of a series of 23 patients treated with 100 mg. of heparin I. V. twice weekly showed eye improvement. This improvement consisted of such definite findings as increased visual acuity, disappearance of retinal hemorrhages, and shrinking of exudates. There were no control patients.

Our evaluation of heparin therapy was carried out on two separate groups of patients with senile macular degeneration. The larger group, consisting of 25 patients, were inmates of an old-folks' home. To be included in this group, the patient was required to have ophthalmoscopically visible senile macular degeneration. This excluded any patients with dense opacities of the clear ocular media. Details of each case are appended at the close of this paper. At the beginning and the end of treatment the study of each patient included corrected visual acuity, central fields, ophthalmoscopic examination, and fundus photography. After initial examination the patients were grouped into pairs whose macular changes were as nearly alike as possible in respect to appearance and extent. These pairs were then divided into heparin and control assignments. Heparin patients received 100 mg. of heparin intravenously twice weekly. Control patients received an identical-appearing saline injection twice weekly. Patients were not aware of the existence of this placebo control.

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An entirely different group, of nine patients, originated from the private practice of several ophthalmologists. These patients also were required to have ophthalmoscopically visible senile macular degeneration. They were assigned to heparin or control groups by simple alternation determined by the time of entering the study. Medication was the same as in the group of 25 patients. These patients were studied a great deal more intensively.

Visual fields and ophthalmoscopic findings were recorded weekly during the first months of therapy, then monthly. Fundus photographs were taken approximately monthly or at any time when visible changes had occurred. Note should be taken of the psychologic effect of a great deal of attention from residents, staff men, and medical students and of the pleasant social aspects of meeting twice weekly with a group of rather nice people suffering from the same disease and shepherded by a very understanding secretary.

Table 1 records the visual changes occurring in the larger series. As can be determined from study of the appended case histories, most of these changes were not of greater magnitude than one or two lines of the chart. It is immediately evident that heparin therapy was associated with improvement in seven eyes and "prevented from becoming worse" in 14 eyes. The sig-

TABLE 1.—*Comparison of Initial and Final Visual Acuity of Each Eye (Group 1)*

	Same	Better	Worse
Heparin	14	7	5
Placebo	10	8	6

TABLE 2.—*Comparison of Initial and Final Acuity of Each Eye (Group 2)*

	Same	Better	Worse
Heparin	6	2	2
Placebo	6	1	1

nificance of this therapeutic accomplishment fades when almost exactly the same results are found in the control group. The majority of these patients recognized no appreciable improvement and derived no psychologic benefit from the injections.

Table 2 records the visual changes in the second group of patients. Again it must be emphasized that these changes were not great. This series is too small to permit conclusions; however, heparin and placebo results were practically identical. The psychological impact of all this medical attention and of the new friendships was remarkable. Excellent morale and optimism pervaded the group far out of proportion to their visual improvement. As recorded in the case histories, specific examples of improvement were cited by many of these patients. Attention is directed to Case 1 (heparin), who was certain she had improved, even though we documented the growth of central scotomata and definite lowering of visual acuity! All but one of these patients were very reluctant to discontinue the study, which had been presented to them as an experimental evaluation of a new treatment reported as helpful in some cases.

Comment

The following general observations are derived from the careful and prolonged study of these 34 cases.

1. Senile macular degeneration is a bilateral disease. Rather often there is marked asymmetry of severity, however, and patients were encountered who had suffered visual loss in the worse eye many years before beginning symptoms were noted in the better eye. Although enormous variations exist in the appearances of senile macular degeneration, there is almost always a close similarity in the degenerative components of the two maculae (e. g., pigment, drusen, choroidal atrophy). Unquestionably, examination of both eyes is necessary in the study and differential diagnosis of macular disorders.

2. Degenerative changes in these eyes were usually not confined to the macula. As compared to aged patients seen primarily for refractive complaints, these patients with macular degeneration suffered considerably more marked general deterioration of the eyes. Circumpapillary chorioretinal atrophy, peripheral pigmentary retinal degeneration, extensive deposits of macular and peripheral drusen, posterior vitreous detachment, and severe arteriosclerotic changes were commonly seen. Advanced cataracts in both eyes were not present in this group because our selection eliminated patients whose fundi were not visible. Several patients were aphakic or had unilateral cataracts, and a considerable number of cases of macular degeneration with associated cataracts had been rejected from the group because it was impossible to photograph their fundi. It is our impression that extensive macular drusen strongly predispose to development of senile macular degeneration.

3. Spontaneous variation in severity of the degenerative lesion is not uncommon. Even under constant conditions of measurement there may be wide difference of visual acuity not obviously related to ophthalmoscopically detectable change. When one examines the records and finds that from month to month visual acuity may improve and then decrease as much as two lines above or below the initial acuity, it is very apparent that our Tables, constructed from initial and final acuity varying as little as one line, are not based on changes great enough really to permit classification as "better" or "worse."

As followed by ophthalmoscopy and retinal photography, some types of degeneration showed no visible changes over a period

of months. This was true of the cases characterized primarily by choroidal atrophy, pigmentation, or drusen formation. In contrast, the disciform types of degeneration (characterized by hemorrhages, soft exudates, neovascularization, and retinal elevations) showed readily observable changes from month to month. Hemorrhages disappear the most rapidly, though new hemorrhages appear elsewhere. Instances of dramatic improvement in acuity undoubtedly occur when a foveal hemorrhage absorbs. Should such a hemorrhage bring the patient to his physician, any therapy (or none) will result in dramatic recovery from "an invariably progressive degenerative disease."

Exudative changes also absorb, though more slowly than hemorrhages. Some degree of scarring and distortion of the retina persists at the site of absorbed lesions. Serous detachments of the central retina may occur in exceptionally severe cases of macular degeneration. Presence of such a detachment cannot, therefore, be construed as indicating by itself that a central lesion is neoplastic.

Neovascularization and microaneurysms may develop in the disciform types of degeneration. Probably some relationship exists between this vascular growth and the hemorrhages which are so common in disciform degeneration. Development and spontaneous absorption of such hemorrhages are partly responsible for the pronounced variations in acuity so characteristic of disciform degeneration.

4. Heparin therapy in the amount of 100 mg. intravenously twice weekly did not alter the natural course of senile macular degeneration. Because of the spontaneous variability of this disease and the psychologic effect of any therapy, no study of a method of treatment can be valid without a placebo control group.

Minor bleeding was not infrequent at the site of heparin injection. This was minimized by aspiration of blood before withdrawal to avoid leaving a track of heparin in the tissues, by use of sharp No. 24 needles, and by prolonged local pressure after injection. Only two patients had systemic bleeding. The worst case developed a fairly large preretinal hemorrhage and repeated hemorrhages into the subdeltoid bursa. These hemorrhages cleared despite continued heparin therapy. Coagulation studies preceding injections were invariably normal.

5. The psychologic effect of enthusiastic and attentive treatment should never be underestimated. Simply the act of paying attention to an elderly deteriorating person is beneficial to him. Meeting new friends with common problems, escaping the "shut-in" home routines, sharing in a research project with enthusiastic physicians, the age-old expectation of benefit from medication, the remarkable adaptability of the human being to his handicaps with time, and probably many other factors contributed to make Group 2 happy. Almost all of this group were reluctant to stop treatment (several continued at their own expense with their private physicians) and were unjustifiably optimistic about the "improvement" of their eyes.

By contrast, the morale of Group 1 was never good. Isolated from the world, infirm and surrounded by the infirm, receiving minimal individual attention, and being a "captive" group rather than voluntarily having sought medical care, these patients tended to be easily discouraged, attributed all types of undesirable symptoms to the injections, and dropped out of treatment prematurely.

We do not endorse giving, placebo-wise, ineffectual medications to the patients with senile macular degeneration. Most patients deserve a frank discussion of the problem with emphasis placed on the good quality of the peripheral vision, which will always be retained. Reference to future deterioration should be minimized as far as possible. Positive therapeutic suggestions should be directed toward the maintenance of general health, e. g., avoidance of overweight, exercise, and balanced diet, not because these suggestions help the eye but because they

give the patient something to do for himself. Prescription of a new pair of glasses which do not significantly improve vision will often produce a most unhappy patient, and the unwary refractionist is hereby cautioned against putting more minus sphere on the patient with advanced macular degeneration and early nuclear sclerosis.

Summary

Thirty-four patients with senile macular degeneration were treated with biweekly injections of heparin and of a placebo.

The naturally variable course of senile macular degeneration was not altered by heparin.

General observations are made regarding the characteristics of senile macular degeneration.

Group 1—Placebo Section

CASE 1.—An 83-year-old white woman noticed marked decrease in vision O. D. in the past six to eight years and less difficulty with O. S. over the past four years. Visual acuity at the beginning of the test period was recorded as light perception O. D. and 20/70 O. S. She was found to have a dense cataract O. D.; O. S. was aphakic, having undergone cataract extraction some six years before. The fundus O. D. was not visible, and O. S. there was a faint horizontal linear hemorrhage in the macula with pigmentary degeneration. On field examination there was a very small central scotoma in the left eye. After 10 weeks of placebo therapy the vision was recorded as light perception O. D. and 20/60 O. S. After 17 weeks of placebo therapy the vision was light perception O. D. and 20/70 O. S. Fundi remained the same, and field examinations revealed no scotomata. Subjectively, this patient was certain that her vision was considerably better and would liked to have continued the "therapy."

CASE 2.—An 83-year-old white woman noted marked decrease in vision O. U. during the past eight years but especially in the past three years. Visual acuity recorded at the beginning of test period was hand movements O. D. and 10/200 O. S. Fundus examination prior to the test period revealed early disciform degeneration with a gray fluffy slightly elevated area in the right macula. There were moderately advanced arteriosclerotic changes. The left macula showed an unusual discretely outlined white infiltrate. Visual-field examination revealed a 5 degree scotoma O. D. and a 2½ degree scotoma O. S. to a 3/1,000 white object. After 12 weeks of placebo the vision was light perception O. D. and counting fingers at 2 ft. O. S. At the end of the test period, 17 weeks, the right fundus was the same as previously, with several small aneurysms noted on the inferior edge of the macula. The O. S. had a less discrete appearance in the macula, with golden glistening reflections. Field examination at the end of the test period showed only a tiny field of vision at the extreme right periphery O. D. and multiple small central scotomata O. S. Vision at this time was hand movements O. D. and counting fingers at 4 ft. O. S. Subjectively, the patient felt that on some days her vision was somewhat improved. However, generally there was not much change.

CASE 3.—An 81-year-old white woman had gradually decreasing vision O. U., especially during the past year. At the beginning of the test period, visual acuity was recorded as 20/100 O. D. and 20/60 O. S. Fundus of the right eye revealed a small macular hemorrhage and vitreous opacities extending forward from the disc. The left macula showed considerable granularity. No scotomata were found. However, both fields were markedly constricted. After 12 weeks of placebo therapy vision O. D. was 20/100, O. S. 20/60+. After 17 weeks the vision was 20/200 O. D., 20/60 O. S. The fundi revealed faint granular irregularity in both maculae. There was noted on this examination an occlusion of the upper temporal vein O. D., which appeared as a white cord, and there were extensive aneurysms temporal to the right macula. Visual field remained the

same. Subjectively, this patient noted no change in her visual status.

CASE 4.—An 83-year-old white man had noticed poor vision O. U., but especially O. D., during the past three-to-four years. Visual acuity at the beginning of the test period was recorded as hand movement O. D., 20/100 O. S. Fundus examination revealed bilateral circumpapillary chorioretinal atrophy. An old hemorrhage was seen in the right macula with a few fine shiny deposits. The left macula was quite indistinct. Visual fields revealed no scotomata in the right eye, but the left eye had a 15 degree central scotoma to 3/1,000 white object. At the end of the test period, vision was still hand movements O. D., and 20/100 O. S. The right macula showed no evidence of the previously seen hemorrhage, and there was indistinct irregularity in both maculae, with slight pigment irregularity. O. D., but O. S. revealed only a 2½ degree central scotoma to a 2/1,000 white object. Subjectively, the patient felt that there was absolutely no change in his visual acuity.

CASE 5.—An 80-year-old white woman noted considerable loss of vision O. U. during the past two years. Vision was 20/300 O. D., 15/300 O. S. Dense brunescent nuclear cataracts were visible O. U. The fundi showed irregular drusen in both maculae, with slight pigment irregularity. No scotomata were plotted. After 12 weeks of placebo therapy vision was recorded as 20/60 O. D., 20/80 O. S. After 17 weeks the vision was 15/100 O. D., 20/100 O. S. Again, no scotomata was found. Subjectively, the patient felt certain that her vision was considerably better than it had been prior to the "therapy" period. N. B., this patient was unable to cooperate well for visual-field examination, hence the fields are not valid.

CASE 6.—An 87-year-old white man had markedly decreased vision O. U. during the past five years, especially the last three. Acuity was recorded as light perception only O. U. Fundus O. D. showed an indistinct gyrate choroidal atrophy with superimposed extensive scarring of old disciform degeneration. The O. S. had similar atrophy but no disciform degeneration. There were small central hemorrhages in both maculae. Visual fields showed only very slight peripheral perception O. D. The O. S. had a small area of temporal vision with no central fixation. After 12 weeks of placebo vision was recorded as counting fingers at 2 ft. O. D. and 5/200 O. S. After 17 weeks it was found to be 3/200 O. D., 3/200 O. S. Fundi at the end of this period remained essentially the same. There was noted some neovascularization in the right macula; there were a few pigment dots in the macular area, with slight elevation, and the edges had a scalloped shape. After this period of placebo administration, the patient felt that his vision was considerably worse.

CASE 7.—A 79-year-old white man noted rather marked decrease in vision O. S. during the past year. Visual acuity at the start of the study was 20/25 O. D., 20/200 O. S. There was irregular pigment distribution in both maculae, a somewhat larger area in the left than the right. There was bilateral circumpapillary chorioretinal atrophy. No scotomata were found even to a 2/1,000 white test object. After 12 weeks of placebo injection, the visual acuity O. D. was recorded as 20/25−2, O. S. 20/100. At the end of the test period, 14 weeks, the vision was 20/30 O. D., 20/100 O. S. The maculae both appeared as before, with the right showing an indistinct area with slight pigment. O. S. was very distinct but with prominent pigment formation. O. D. showed no scotoma, but O. S. had a central 2½ degree scotoma to a 3/1,000 white test object. N. B., this patient had placebo therapy for only a total of 14 weeks. The patient felt that at the end of the period he had not improved and was probably worse. He had refused to continue the injection after 14 weeks, owing to his feeling of lack of improvement.

CASE 8.—This was an 87-year-old white woman who had noted slight decrease in visual acuity O. U. during the past year. Visual acuity was recorded as 20/40 O. U. at the beginning of the test period. The

fundi revealed many large drusen about both maculae, with slight pigmentary deposits within the maculae and rather advanced choroidal sclerosis, leaving very indistinct foveas. No scotomata were found on field examination. After 17 weeks of placebo, the vision was recorded as 20/70 O. D., 20/50 O. S. There was a 1 cm. central scotoma found O. D. to a 3/1,000 white test object. No scotoma was found O. S. Maculae remained unchanged O. U. Subjectively, this patient felt there had been no change whatsoever in her visual status.

CASE 9.—An 83-year-old white woman noted gradual decrease in vision O. U. but more marked O. S. during the past one and one-half to two years. Visual acuity at the start of the test was 20/100 O. D., 20/200 O. S. After 12 weeks of therapy the vision was recorded as O. D. 20/70, O. S., counting fingers at 2 ft. Fundus examination revealed minimal atrophy with moderate pigment proliferation in both maculae, but the left more than the right. Central fields revealed a 5 degree central scotoma O. D. and none O. S. to a 3/1,000 white test object. At the end of the test period, the scotoma O. D. was about 6 degrees to 5/1,000 white test object. The O. S. at this time revealed a 10 degree scotoma to a 3/1,000 white test object. This patient felt that her vision was considerably improved O. U. after receiving therapy. Her visual acuity at this time, however, was 20/100 O. D. and counting fingers at 2 ft. O. S.

CASE 10.—An 83-year-old white woman had complained of moderate loss of vision O. U. during the past three years but more marked O. S. during the past year. Visual acuity at the beginning of the test was 20/100 O. D., 20/200 O. S. Fundus examination showed a slightly elevated 2 D. D. area, with many drusen, some slight pigment, and a few microaneurysms in each macula. There were also central macular hemorrhages in the right and two paramacular hemorrhages in the left macula. After receiving placebo for 17 weeks, the vision was recorded as 70/70 O. D., 20/200 O. S. Early nuclear cataracts were noted at this time, and both macular regions were very indistinct, owing to slightly raised edematous lesions. There were still several small aneurysms O. D. No hemorrhages were seen O. S. No scotomata were found in either eye before or after the test period. Subjectively, the patient felt that there had been no change in her vision.

CASE 11.—A 74-year-old white man had noted gradual slight decrease in vision O. U. during the past year or two. Visual acuity was recorded as 20/40− O. U. at the beginning of the study. The maculae showed no foveal reflex, with slight irregularity in the pigment, and there were two pinpoint shiny deposits in the right macula. No scotomata were found. After 10 weeks of therapy the vision was recorded as 20/50+1 O. D., 20/60−1 O. S. After 17 weeks of placebo the visual acuity was recorded as 20/30− O. U. The fundi remained unchanged, and again no scotomata were found in either eye. Subjectively, this patient felt that there had been no change in his eyes.

CASE 12.—An 88-year-old white woman noted considerable loss of vision O. D. during the past eight years and less marked loss O. S. during the past year. Vision in the right eye was light perception and 20/100 O. S. There was a very dense cataract O. D. The fundus O. S. revealed circumpapillary atrophy with poor macular definition. The left eye was aphakic. The right macula was not visible. There were no scotomata found. After 17 weeks of placebo, vision remained as light perception O. D. and 20/200 O. S. Subjectively, there was no change.

Group 1—Heparin Section

CASE 1.—An 86-year-old white man had poor vision O. S. for some years and recently complained of slight decrease in vision O. D. Visual acuity was 20/40 O. D., hand movements O. S. The left fundus was not visible, owing to a dense cataract. The right fundus showed absence of the foveal light reflex. The fields were normal O. D.,

but there was a 5 degree central scotoma in the left to 5/1,000 white test object. At the end of the heparin administration, vision remained the same but no scotomata were found. Fundus was unchanged. Subjectively, he felt there had been no improvement.

CASE 2.—A 76-year-old white woman complained of failing vision O. U. for the past 10 years, with marked loss in the last 2 years. Initial vision was recorded as 18/200 O. D., 4/200 O. S. There was a small central scotoma O. U. with 5/1,000 white test object. The fundus revealed atrophic maculae with scalloped pigmented margins. Vision after 10 weeks was 18/200 O. D., 8/200 O. S. The fundi appeared the same as at the beginning of the test. There was a definite increase in the size of the scotomata O. U. This patient had considerable hematoma formation after each injection of heparin, followed by severe pain from the elbow to the shoulder. Consequently, she only underwent 10 weeks of therapy. Subjectively, at the end of the testing period she felt that her eyes were somewhat worse than at the start.

CASE 3.—An 87-year-old white man had decreasing vision O. U. for the past 10 years. Visual acuity at the start of the test period was 4/200 O. D., 20/100 O. S. There was an indefinite scotoma O. D., varying from 2½ to 10 degrees. There was a definite 2½ degree central scotoma to 3/1,000 white object O. S. There were atrophic areas with small drusen and multiple small pigment clumps in both maculae. After five weeks of therapy, vision was recorded as 20/100 O. U. At this time, the central scotoma O. D. was very definite and there was a variable 5 degree scotoma O. S. Subjectively, this patient felt that during the heparin-testing period his vision had become considerably worse. This patient only completed five weeks of therapy because of severe local hematomas despite very careful injection.

CASE 4.—An 88-year-old white woman had decreasing vision over the past several years O. U. Initial acuity was 15/100 O. D., 20/100 O. S. A small central scotoma was found O. D.; none, O. S. Fundus examination showed poorly defined maculae with loss of detail O. U. After 10 weeks of therapy vision was 20/60−1 O. D., 20/40−3 O. S. After 17 weeks of therapy the vision was 20/80 O. D., 20/40 O. S. At the end of the examination, no scotomata were found in either eye. This patient felt that she had some slight improvement during the period she was on medication.

CASE 5.—An 82-year-old white man noted considerable loss of vision in both eyes over the past 15 years, more marked in the left eye, especially in the last five years. Visual acuity was recorded as 20/70 O. D. and light perception O. S. Fundus examination revealed poor macular definition, with no foveal light reflex O. D. The left fundus was not visible, owing to an opaque lens. Visual-field examination was unsatisfactory in this patient, owing to the patient's lack of understanding of the test. After 10 weeks of therapy vision was recorded as 20/100−1 O. D. and light perception O. S. After 17 weeks of therapy vision was 20/70−1 O. D. and light perception O. S. Subjectively, this patient noted no change in his vision during the period of heparin administration.

CASE 6.—An 85-year-old white woman complained of gradually decreasing vision O. U., more marked O. S., during the past several years. Visual acuity O. D. was 20/40, O. S. 20/200. Fundus examination revealed rather marked choroidal sclerosis with loss of pigment in both macular areas and a few small areas of pigment proliferation. No scotomata were found on the field examinations. At the end of the period of receiving herapin, her vision O. D. was recorded as 20/50; O. S., as 20/100. Fundus and visual-field examinations remained exactly as before. Subjectively, this patient felt sure that her vision was markedly improved.

CASE 7.—A 79-year-old white man noted considerable loss of vision, especially O. D. but present O. U., over the past 12 years, more marked in the last three years. Acuity

at the initial examination was 20/400 O. D., 20/100 O. S. Fundus examination revealed many very large drusen about both maculae. Central scotoma could not be demonstrated. At the end of the test period the vision was 20/100 O. D., 20/80−2 O. S. Fundus and field examinations remained unchanged. Subjectively, this patient noted no change in his vision during this time.

CASE 8.—An 84-year-old white man complained of visual difficulties O. U. during the past 10 to 15 years. Visual acuity prior to receiving heparin was recorded as 3/200 O. D. and light perception O. S. The right macula revealed a proliferative lesion elevated about 2 D. There was marked choroidal sclerosis. The left fundus was not visible, owing to a dense cataract. Visual field revealed a somewhat variable 5 degree central scotoma on the right; the left could not be plotted. After both 10 and 17 weeks of therapy, visual acuity was O. D. counting fingers at 6 in., O. S. light perception. The proliferative lesion did not appear quite so elevated, and there appeared to be better central fixation on the visual-field examination, with no central scotoma to 5/1,000 white object. The patient could not see a 3/1,000 white object. Subjectively, his vision seemed worse.

CASE 9.—A 79-year-old white man noted considerable loss of vision O. D. during the past year, with slight decrease in acuity O. S. during the last several months. At the beginning of the test period, his vision O. D. was recorded as 20/100; O. S., 20/30+. Fundus examination revealed minimal atrophic degeneration O. D., with pigment stippling O. U. There was a 7 mm. scotoma centrally O. D. to a 3/1,000 white test object. This could not be found with a 5/1,000 white test object. O. S. had no scotoma. After 10 weeks of therapy the visual acuity was recorded as 20/100+2 O. D., 20/25−2 O. S. After 17 weeks the vision was 20/70 O. D., 20/25− O. S. Fundus examination at this time was unchanged, except pigmentation seemed less marked. The same scotoma was found O. D.; none, O. S. Subjectively, this patient noted no particular change in vision.

CASE 10.—An 83-year-old white man noted the onset of decreasing vision O. U. some 12-to-15 years previously, recently much more marked O. D. Prior to the administration of twice-weekly heparin, his visual acuity was found to be 20/200 O. D., 20/60 O. S. Fundus examination revealed dull irregular maculae with several small hemorrhages. No definite scotomata were found on field examination; however, there was one small central scotoma O. D., which was extremely variable but almost constantly present. After 12 weeks of therapy the vision was 20/200 O. D. and 20/30 O. S. After 17 weeks the visual acuity was found to be 12/200 O. D., 20/60 O. S. No hemorrhages were found on this examination. Markedly advanced arteriosclerotic changes were noted. On this examination, no scotomata were found. Subjectively, this patient felt that his vision O. U. was considerably worse.

CASE 11.—A 72-year-old white woman noted slight loss of vision over the past year. Visual acuity prior to receiving heparin was 20/40 O. D., 20/50 O. S. Fundus examination revealed loss of macular definition. There was increased luster around the right macula and a very fine hemorrhage with some stippling in the left macula. Angioid streaks were noted in both eyes, and in the right eye there was a small circumpapillary hemorrhage. After 10 weeks visual acuity was recorded as 20/30−2 O. D., 20/40+4 O. S. At the end of the test period the visual acuity was 20/30−1 O. D., 20/50 O. S. Visual-field examination, done prior to receiving heparin, revealed no scotomata. At the end of the test period, there was found with a 3/1,000 white object an inferior temporal pie-shaped sector defect O. S., sparing the macula. Fundus examination at the end of the test period was the same as previously, with the addition of a visible macular cyst on the right. At the end of this period, this patient felt that her vision was subjectively not nearly as clear as prior to receiving heparin.

CASE 12.—An 86-year-old white man received only five weeks of therapy, after which he refused further injections. Visual acuity at the beginning of the test was 16/200 O. D., 20/100 O. S. At the end of the period, it was recorded as 10/200 O. D., 15/200 O. S. Fields were not possible on this patient. Fundus examination revealed complete punched-out choroidal atrophy, with no elevation or pigment visible in the macular area. This patient felt that his vision was considerably worse under treatment than before.

CASE 13.—A 70-year-old white man had poor vision O. D. for the last several years. Visual acuity at the beginning of the test was counting fingers at 2 ft. O. D., 20/40+2 O. S. Fundus examination revealed many large macular pigment deposits with drusen and atrophy in both eyes. The O. D. showed an old occlusion of both upper temporal arteriolar branches, with sheathing of these vessels causing a large central scotoma to 10/1,000 white test object. Visual acuity after 15 weeks of therapy, which is all this patient received, was counting fingers O. D. at 2 ft. and 20/40 O. S. Fundus examination remained exactly the same. This patient felt that his vision was considerably improved, especially that the blind spot next to his nose was smaller and fainter.

Group 2

CASE A.—This patient was a 77-year-old white woman whose left eye had been bad for 18 years and whose right eye was poor for four or five months. Vision corrected to 20/50 O. D. and counting fingers at 1 ft. O. S. The right macula contained an indistinct degenerative area 2 D. D. across, including a rough circinate figure. Below disc and macula was a 4×8 D. D. degenerative area confluent with the macular degeneration. This lower area contained several discrete circinate figures, several moderately large preretinal hemorrhages, and areas of exudative retinal elevation. The left macula was completely included in a sharply punched-out flat atrophic chorioretinal defect, which permitted a clear view of underlying large choroidal vessels. No exudates or hemorrhages were present O. S. There was marked circumpapillary chorioretinal atrophy O. U. One-third of the central-field area of 30 degrees radius was obliterated O. D. and about one-quarter of this area, O. S.

Placebo injections were given twice weekly from July 5 to Dec. 13, 1957. Eleven fields showed minor variations of the extensive scotomata described above. Visual acuity at the end of therapy was 20/50 O. D. and counting fingers at 1 ft. O. S. The left fundus did not change during observation. The right lesion underwent variations in degree of elevation, location and extent of hemorrhages, and distribution of exudates.

The patient noted no improvement in vision, suffered from many physical infirmities, and was generally discouraged.

CASE B.—A 65-year-old white woman had noted blurred vision O. S. for one year and O. D. for six months. She could see no better than to count fingers at 3 ft. O. S. and was correctible to 20/50 O. D. She had massive disciform degeneration O. U., worse O. S. These lesions were from 5 to 8 D. D. in extent and contained circinate retinopathy, extensive preretinal hemorrhages, microaneurysms (patient not diabetic), exudates, elevated retina due to exudative detachment, and chorioretinal atrophy. Tangent-screen examination with 5/1,000 white object showed extensive loss, the scotoma involving almost 1/3 of the central 30 degrees radius O. D. and more than 2/3 of this area O. S.

Heparin, 100 mg., was given intravenously twice weekly from July 5 to Dec. 13, 1957. Two weeks after start of therapy she developed a massive preretinal hemorrhage occupying about 1/3 of the inferior peripheral retina. Therapy was not interrupted, and in two and one-half months this large hemorrhage had disappeared except for scattered deposits of pigment. During the treatment period smaller retinal hemor-

rhages constantly appeared and disappeared. This patient had three episodes of bloody effusion into her subdeltoid bursa, proven through aspiration by an orthopedic surgeon. Ten fields showed little variation beyond that expected from poor central fixation. The last acuity was 20/50 O. D. and counting fingers at 6 in. O. S. Rather definite fundus changes occurred throughout observation, with hemorrhages of varying size appearing and gradually absorbing, tending to be replaced by exudative or scarred deposits. Rather pronounced serious detachment of portions of the central retina occurred transiently. Soft exudates varied slowly over a period of months, and hard exudates seemed almost unchanged during the five months of observation.

Subjectively, the patient noted no recovery of vision but expressed willingness to continue therapy indefinitely.

CASE C.—This was a 52-year-old white woman with two years' gradual reduction of vision O. S. Initial corrected acuity was 20/30 O. D., 20/200 O. S. The right macula contained a scattering of drusen and small pigment dots surrounding a 0.25 D. D. central pigmentation. The left macula was essentially similar, except for the addition of a 1.5 D. D. flat central depigmented atrophic area. Surprisingly, only a 2 degree (1/1,000 white object) scotoma was demonstrable O. S.

Placebo injections were given twice weekly from July to Dec. 13, 1957. A total of nine visual fields were done. The small central scotoma was recorded O. S. only three times, initially, terminally, and once toward the end. It was not possible to recognize any change of either macula during the period of observation. Final acuity was 20/30 and 20/200.

She reported making fewer clerical errors at the end, though admitting that this might be due to more care in her work. She thought vision seemed slightly better but was not completely sure.

CASE D.—A 62-year-old white woman had noted two months' decrease of vision O. D. Initial corrected acuity was 20/50 O. D. and 20/20 O. S. In the right macula was an elevated whitish mass about 1 D. D. across, covered with scattered pigment granules and surrounded by many small drusen. The left macula was similar, except that the mass was less well demarcated and not elevated. Multiple pigmented drusen were situated in the peripheral retina, and a small posterior vitreous detachment existed.

The initial visual field showed a 10 degree central scotoma with 1/1,000 white object O. D., no scotoma O. S.

Heparin, 100 mg., in intravenous injections, was given twice weekly from July 19 to Dec. 13, 1957. Ten fields were done, and all but two showed a central scotoma of variable size, O. D. Three showed a tiny paracentral scotoma O. S. Visual acuity O. D. varied from 20/100 to 20/40. The field defects and acuity did not change consistently but randomly, and they could not be said to prove progression or regression of the defect. Final acuity was 20/40 and 20/20. Her subjective report was that her vision seemed the same in all respects, neither better nor worse.

CASE E.—An 84-year-old white woman had reduced vision of one year's duration. Acuity O. D. was counting fingers at 1 ft.; O. S., 20/100 corrected. This patient had most severe retinal and choroidal arteriosclerosis. The right macula was obscured by hazy grayish circular infiltrates about 1 D. D. across. Scattered pigment irregularity also was present in the macula. Several dozen tiny-to-small hemorrhages were spread throughout the posterior pole. The left macula contained marked pigment stippling and irregularity. A single 0.1 D. D. superficial retinal hemorrhage lay between the left disc and macula. Fields with 3/1,000 white object showed a 30 degree central scotoma O. D. and a 15 degree central scotoma O. S.

Biweekly placebo injections were given intravenously from Aug. 2 to Oct. 11, 1957. She did not continue because of a broken leg. Five fields showed no significant change. Final acuity was O. D., counting fingers, and O. S., 20/200. The ophthalmo-

scopic appearance did not change during this two-month period.

The patient did not report subjective change.

CASE F.—A 77-year-old white man had decreased acuity for five months O. U., correctible to 20/30+ O. U. Both fundi showed severe choroidal and retinal arteriosclerosis, with marked pigmentary irregularity and stippling of both foveas (about 0.5 D. D. area). The right lesion was more extensive, and peripheral to it was a small hemorrhage and three small areas of well-defined choroidal atrophy. The initial visual field was recorded as showing a 15 degree paracentral scotoma to 2/1,000 white object O. U.

Heparin, 100 mg., was given intravenously twice weekly from Sept. 20, 1957, to March 7, 1958. Six visual fields were recorded, showing no significant change, O. D. Inexplicably, the large scotoma O. S. was never found after the initial field. The second field, done only two weeks after the first, showed no trace of this scotoma. Visual acuity varied widely, once being as low as 20/200 O. D. and 20/50 O. S. The last recorded acuity was 20/30− O. U. Ophthalmoscopy showed complete disappearance of the small hemorrhage O. D., though the involved macular area increased slightly in size during observation.

The patient reported considerable day-to-day variation in acuity, but he was convinced that he could read better at the end of therapy. He recognized clearly that "the psychological effect has been tremendous" and said he had been most discouraged when first diagnosed as having intraocular hemorrhage and degeneration.

CASE G.—This was a 65-year-old woman whose left eye lost vision "40 years ago." The right eye went bad "nine months" before examination (she said, though photographs were available which showed O. D. to have had an exactly identical appearance more than a year previously). Acuity was counting fingers in distance O. D., counting fingers at 1 ft. O. S. The right macula was crossed by a very discrete curved band of tissue of 1×3 mm. size. O. S. had a typical disciform degeneration about 5 D. D. across, with a circinate figure, several large hemorrhages, flat detachment of the central retina, and scattered exudates. Field with 3/1,000 white object showed a 15 degree central scotoma O. D., 25 degree, O. S.

Placebo injections were given biweekly from Sept. 6, 1957, to March 7, 1958. Seven fields showed no significant changes. Final visual acuity was O. D. 20/200 questionable, O. S. counting fingers. The right macular lesion was absolutely unchanged during the period of observation and was exactly similar to the year-old photograph. Microaneurysms, hemorrhages, and exudates varied slightly during observation O. S., though the general picture of disciform degeneration remained about the same.

The patient was certain that her vision had been helped and cited specific instances, such as cooking and watching television, where she could see better. She stated she could not see straight ahead initially O. D. but could at the end.

CASE H.—A 69-year-old white woman had had childhood strabismus and never had good acuity O. D. For one year O. S. had failed. Acuity was corrected to O. D. counting fingers at 1 ft., O. S. 20/50+. Both maculae were heavily spotted with drusen and showed hazy central infiltrative changes. An old occlusion of the upper temporal vein O. S. was recognizable through characteristic tortuosity and branching. Posterior vitreous detachment was present. A 10 degree paracentral scotoma was present O. D. to 1/1,000 white object, but no scotoma was recorded O. S. initially.

Heparin, 100 mg., was given intravenously biweekly from Sept. 27, 1957, to March 11, 1958. Of the six recorded fields, three report a 5 to 10 degree central scotoma O. S. to 1/1,000 white object. Because of very poor fixation, the right scotoma was extremely variable. Final acuity was O. D. counting fingers and O. S. 20/30. The fundus O. D. definitely deteriorated, with development during observation of disciform changes and marked overlying venous tor-

tuosity. The left fundus, including the old venous occlusion, did not change.

This patient was most enthusiastic about therapy, emphasizing that it definitely improved vision, helped her "heart condition," "stopped bones from aching," made her more vigorous, caused a gain of 10 lb. (she was a rather thin woman), and made her feel better all over. She thought getting out of her home had helped very much, and since treatment she had taken jobs baby-sitting, caring for a house, and working in a hardware store!

CASE I.—A 70-year-old white woman had visual loss O. S. of several years' duration. Initial corrected acuity was 20/20 O. D., 20/50 O. S. The left macula was slightly elevated and indistinct, with scattered deeply situated pigment flecks visible by proximal illumination. The involved area was about 1.5 D. D. across. Similar changes were present O. D. but were less pronounced and devoid of pigmentation. Senile vitreous liquefaction was conspicuous. No scotomata were demonstrable in either eye.

Heparin, 100 mg., was given intravenously twice weekly from Oct. 4, 1957, to March 7, 1958. Five visual fields were recorded (monthly) and showed gradually evolving central scotomata, which grew larger and denser on each examination. At the time of final field, the 2/1,000 white object defect encompassed a 7 degree paracentral circle O. D. and a 10 degree defect O. S. Visual acuity also declined and was last recorded as 20/40 O. D., 20/50 O. S. The ophthalmoscopic picture became slightly worse O. U., with more elevation and distortion than in the beginning.

Despite this clearly documented deterioration of the eyes, this patient was most enthusiastic about her results and was convinced that she had obtained visual improvement.

Fundus photographs were taken during the course of this study by Prof. Jack Prince. Drs. Makley, Andrew, Sage, Bontley, Magnuson, and Stine permitted use of their private patients. Part of the heparin used in this study was given by the Darwin Laboratories, Los Angeles.

Department of Ophthalmology, The Ohio State University (10).

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1. Rome, S.: Heparin in Senile Macular Degeneration: Preliminary Report, A. M. A. Arch. Ophth. 57:190-199 (Feb.) 1957.

Artifacts and Normal Variations in Human Ocular Electromyography

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Sir Francis Walshe, in discussing "The Future of Neurology" at a meeting of the Royal Society of Medicine, stated his conviction that "a fruitful future for neurology lay rather with experimental pathology than with electrophysiological studies. In the case of the latter, it seemed that many studies revealed more about the properties of the apparatus used than of the functions of the nervous system." [1]

Thus, whenever a relatively new technique involving complicated apparatus, such as ocular electromyography, is introduced, one must establish normal ranges for proper interpretation of the results. More important, one must be able to distinguish artifacts from meaningful data.

A previous publication [2] outlines the technique of human electromyography. The purpose of this paper is to point out (*A*), normal variations of ocular electromyography, and (*B*), artifacts in electromyography. Because of these normal variations and artifacts, we shall, in our discussion, point out some limitations of interpretation and what may be some erroneous concepts based upon artifacts.

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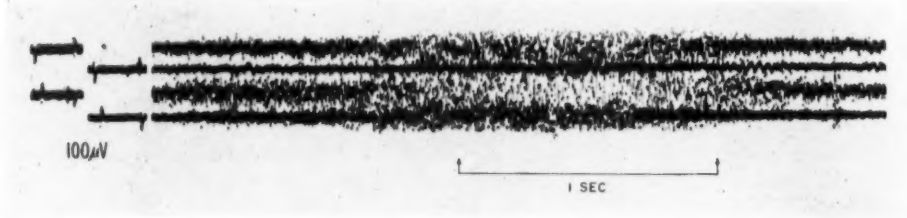
Normal Variations

1. *Degree of Activity.*—The degree of activity recorded depends upon the site of the electrode tip within the muscle. There is a greater recorded activity from the midbelly portion of the muscle than from more distant areas. Different sites within the muscle show large differences in activity.

Figure 1 shows three electrodes at different sites in the same muscle during contraction of the muscle. Electrodes closer to the muscle belly record activity sooner and reach larger amplitudes than the recordings from electrode tips more distant from the muscle belly. With intentionally poor insertions of the electrodes near the tendonous portion of the muscle, the activity is recorded later and reaches smaller maximum amplitudes. Thus, sampling differences may account for marked differences in amplitude, and this is an important reason why relative strengths of muscle contraction may not be

Fig. 1.—Simultaneous recording from three electrodes at different sites within the same medial rectus muscle. Note the differences in time of onset of recording from the different electrode insertions. The second channel is not working.



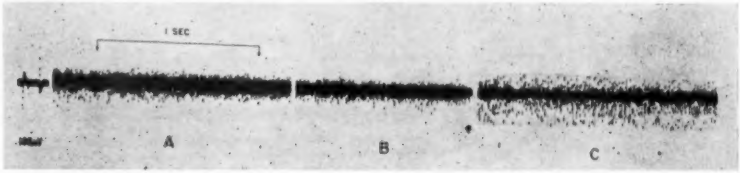


Fig. 2.—Recording from an electrode insertion into the region of crossing of the inferior rectus and inferior oblique muscles. *A*, down-gaze, showing activity recorded from the inferior rectus. *B*, primary position, showing decreased activity recorded. *C*, up-gaze, showing increased activity recording from inferior oblique muscle.

inferred from electromyographic data except in frank, marked, neurogenic disease, as will be pointed out in the discussion. In our experience, electrode placement into the belly of the medial rectus is easier than placement into the lateral rectus, with consequent greater amplitudes of recording from the medial rectus during ordinary testing procedures. Thus, a diagnosis of "spastic esotropia" based upon electromyographic recording of large amplitudes from the medial rectus could be based on merely a normal variation.

Relative changes of activity from the same muscle during contraction are more difficult to observe in recordings with low amplitude of activity. This may be because of a poor insertion or because of minimal activity from a muscle in a field of gaze where minimal contraction takes place.

2. *Activity from More than One Muscle.*—Activity from more than one muscle may be picked up through a single electrode insertion if the concentric electrode shaft or tip rests close to the muscle belly of each. In practice, this is restricted to the instance of an electrode inserted into the area where the inferior oblique and inferior rectus muscles are in juxtaposition near the ligament of Lockwood. Figure 2 is an example of this, in which there is increasing activity recorded in down-gaze (Fig. 2*A*) because of inferior rectus activity. The recorded activity decreases with elevation of gaze to the primary position (Fig. 2*B*). The activity again increases in up-gaze, owing to recorded activity from the inferior oblique muscle (Fig. 2*C*). Evidence that this inference is correct is given in Figure 3, which shows the record from the same electrode tip, which has been slightly shifted during this same recording so that the inferior oblique alone is now recorded. The electrode tip is now at some distance from the inferior rectus, and only the inferior oblique is being recorded, showing the characteristic increase in electrical activity from down-gaze, where there is minimal activity of the inferior oblique (Fig. 3*A*); to the primary position (Fig. 3*B*), where there is an increase in activity; to the up-gaze (Fig. 3*C*), where there is maximal activity. This response of recording from two muscles with a single electrode is similar to, if, indeed, not the same as, examples given by others of "anomalous innervation," as will be discussed later.

3. *Electrode Movement.*—Electrode movement may occur as a result of muscle contraction, although ordinarily its position seems to remain remarkably constant.[2]

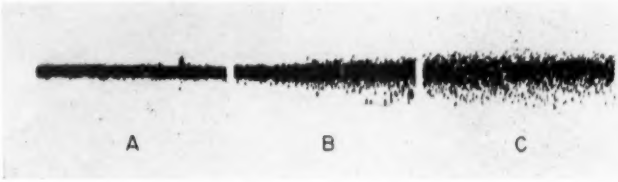


Fig. 3.—Same recording as Figure 2, after a slight change in electrode position. *A*, down-gaze, showing minimal recorded activity of inferior oblique. *B*, primary position, showing gradual increase of recorded activity from the inferior oblique. *C*, up-gaze, showing maximal recorded activity from the inferior oblique. (The time scale and the calibration are the same as in Figure 2.)

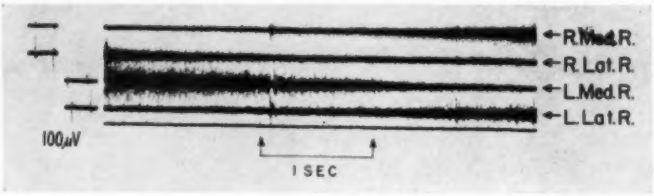


Fig. 4. — Simultaneous recording from the four horizontal rectus muscles during a following movement from right to left gaze. Note the overlapping of recorded reciprocal activity in the two lower channels compared with less overlapping of the two upper channels, owing to sampling differences.

We deduce that if the electrode is placed so that the tip rests near the surface of the muscle, the tip may, in fact, record activity from within the muscle in one position of gaze, while in another position of gaze the tip may work free of the muscle tissue and presumably rest a few millimeters from the tissue and thus record little or no activity. The evidence for this uncommon effect is that the activity may be heard over the loud-speaker to reach a maximum, and during further contraction of the muscle, when the gaze continues to be directed further in the same direction, the activity may be heard to drop off at a certain point in a regular repeatable fashion, presumably as the tip becomes displaced from the muscle tissue. Readjusting the tip so that it very likely rests now within the muscle tissue results in a resumption of the unit activity which can be heard and recorded. Experience indicates that this occurs very infrequently. Electrode movement per se, unless the electrode is inserted further or withdrawn, results in no appreciable change in the recording.

4. *Measured Time Relationships of Electrical Activity Between Muscles.*—These depend upon the relative amplitude of activity in the recordings. Figure 1 shows that the beginning of observable activity in the tracing depends upon the electrode site within the muscle. Figure 4 shows recordings from the four horizontal rectus muscles and demonstrates such sampling differences. There is overlapping of reciprocal innervation of the left medial and lateral rectus muscles because of relatively good insertions, while the right medial and lateral rectus muscles show less overlapping of the reciprocal innervation recordings, simply because of sampling differences.

5. *Other.*—Normal variations in recorded electrical activity from muscles acting in planes going through the primary position [3] and planes between the visual lines in two tertiary positions [4] are discussed elsewhere.

Artifacts

Artifacts interfere with the recording of electrical-unit activity from the extraocular muscles. An artifact may be defined as any extraneous recorded potential. They may arise from several sources, such as the recording of extraneous electrical phenomena, improper shielding of the equipment and cables, and electrical limitations within the equipment.

1. *Television Artifacts.*—Television artifacts may be inadvertently recorded even with good shielding and careful engineering, especially in metropolitan areas where powerful transmitters are located nearby. Figure 5*A* shows a normal subject with unit

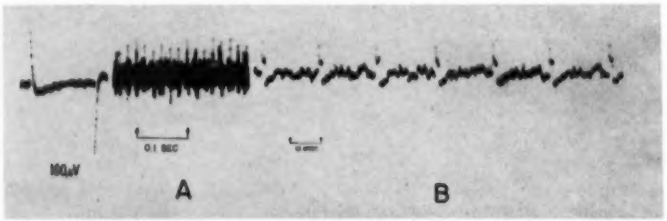


Fig. 5.—*A*, television artifacts superimposed upon normal unit activity at slow film speed. *B*, same as *A* at high film speed. Note the regularly recurring high-amplitude television pulses.

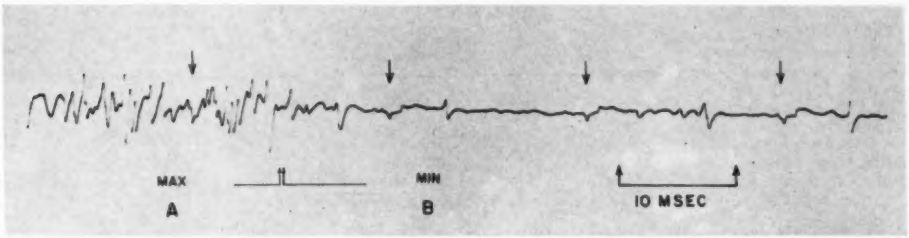


Fig. 6.—A regularly recurring television artifact (arrows) during maximal, *A*, and minimal, *B*, muscle contraction.

activity recorded along with a regularly recurring television artifact. Figure 5*B* is the same as Figure 5*A*, displayed with a higher film-sweep speed in order to observe the wave form of each unit. The television artifact shows a very characteristic pattern.*

Figure 6 shows a television artifact in the recording from a normal subject during maximal (Fig. 6*A*) and minimal (Fig. 6*B*) contraction of the muscle. One notes that the recognizable regularly recurring television artifact decreases in amplitude along with the decrease in actual muscle-unit activity. The fact that an artifact may itself be altered in amplitude along with the recording of actual unit activity indeed poses an ofttimes confusing problem.

Integration of the electrical activity is of little aid, since artifacts are integrated together with actual unit activity.

Such artifacts, recorded alone, may simulate unit activity, since they may change amplitude in a regular fashion with eye position, as does regular unit activity. As will be pointed out subsequently, a poor electrode insertion with low amplitude recording is particularly prone to demonstrate such artifacts.

Further knowledge of such extraneous electrical phenomena may be obtained by a study of television pulses following complete induced paralysis of the muscle and complete cessation of all unit activity following procaine injection. Figure 7 shows a recording from a normal medial rectus during minimal and maximal contraction activity. One will note that the television artifact (arrow), seen clearly during minimal activity (Fig. 7*A*), is masked during maximal activity (Fig. 7*B*) by the normal unit activity. Figure 8 is the same electrode insertion after procaine injection into the muscle and cessation of all unit activity. Figure 8*A* shows the regularly recurring television artifact during minimal contraction and minimal activity (as in Fig. 7). Figure 8*B* shows that during *attempted* gaze into the field where maximal contraction of the muscle would take place the television artifact is observed to increase in amplitude. There is a complete absence of unit activity because of the procainization, but the eye

* These pulses may be identified as TV pulses, vertical synchronizing and blanking, by their characteristic wave form, time duration (1,250μsec.), and frequency (60 pulses per second). They result from TV signals entering the apparatus and are eliminated by proper shielding and grounding.

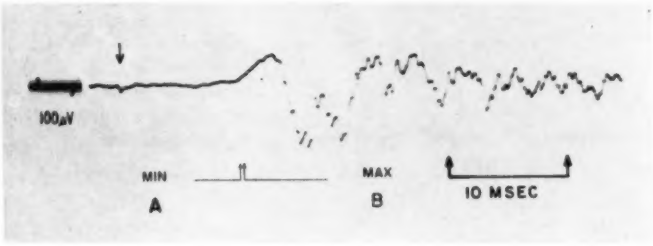


Fig. 7.—A television artifact (arrow) seen clearly during minimal activity, *A*, which is masked during maximal activity, *B*.

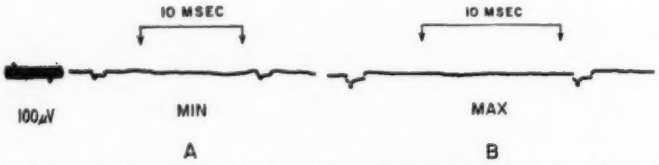


Fig. 8.—Same as Figure 7, following procainization of the muscle, with cessation of all unit activity. Note the increase in amplitude of the regularly recurring television artifact from minimal activity, *A*, to attempted maximal gaze, *B*, into the field of action of the procainized muscle. Although the film speed is increased, as indicated, the voltage calibration is unchanged.

was seen to move less than 10 degrees because of the change of activity of the other associated oculorotary muscles.

Figure 9 shows a slow (*A*) and high (*B*) film-speed "doublet" artifactual activity recorded from an electrode placed in isotonic saline solution without a subject. These spikes represent the characteristic television 60-cycle pulse, which may be mistaken for unit activity.

It should be emphasized that high-film-sweep-speed samples of individual units seen on slower-speed records are necessary for proper analysis and interpretation.

Such television artifacts may be similar to some of the evidence given in support of "anomalous innervation," as discussed later.

2. *Artifacts of Base-Line Movement.*—The character of base-line thickness, composition, and movement has been outlined previously.[2] Some relatively slow shifts in the base line may occur at the beginning or end of any eye movement. Actual unit activity may or may not be superimposed upon the shifted base line. One must be cautious not to interpret such a slow shift in base line as unit activity.

With any quick eye movement, one may record a relatively slow shifting in base line which, as an artifact, may simulate unit activity. At the beginning of a saccadic (quick) eye movement, one does record an actual burst of unit activity in the agonist. This burst of activity, however, must be differentiated from a slow artifactual shifting of base line which may occur. Once again, the electromyogram at fast-camera sweep speed allows one to differentiate the actual composition of the "burst" as artifact versus true unit activity.

The slow artifactual deflections of the base line which occur at the *beginning* of a quick eye movement may also occur in the completely inhibited antagonist in its inhibitional phase during the movement in the opposite direction. Obviously, such deflections of the base line do not represent a burst of real unit activity taking place during the inhibitional phase but must be artifactual. Grossly, however, the artifactual shifting of the base line may be similar to that which may occur at the beginning or cessation of true unit activity with change in eye position. Figure 10*A* shows recruitment during a small quick change in eye position with

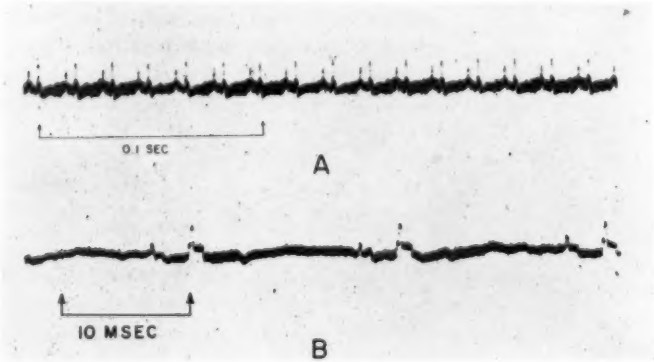


Fig. 9.—"Doublet" artifactual activity recorded from an electrode placed in isotonic saline solution without a subject at normal film speed, *A*, and high film speed, *B*.

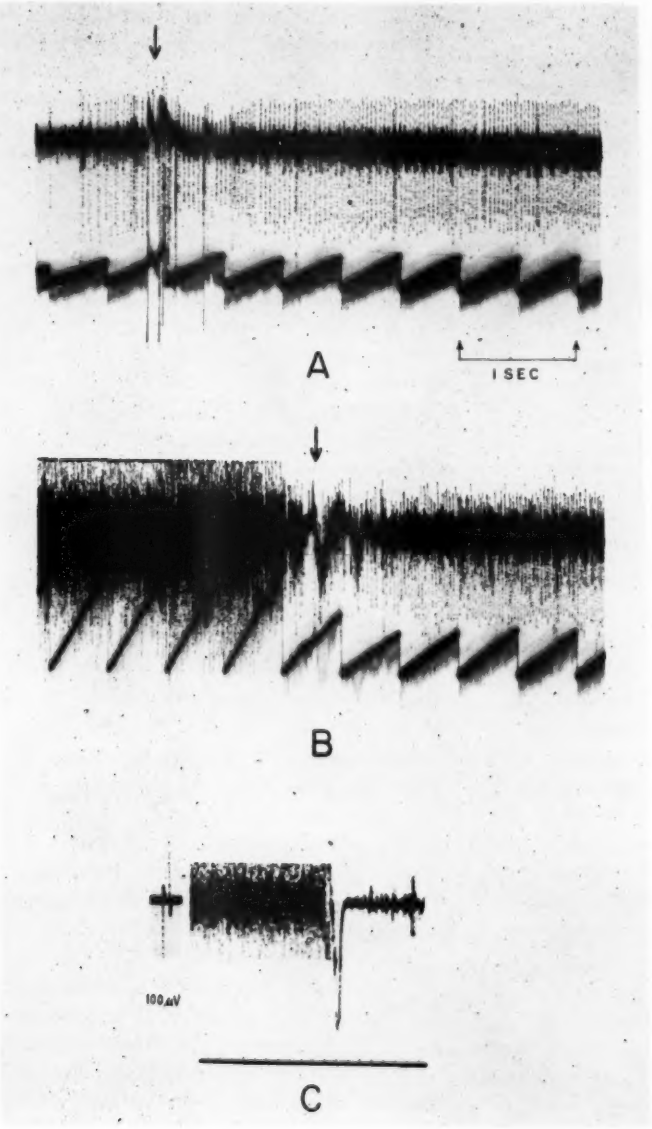


Fig. 10.—*A*, artifactual deflection of the base line during recruitment phase of a small quick change in eye position (arrow). *B*, a similar artifact with a quick change in eye position in the opposite direction (arrow). *C*, the shifting of the base line at the start of an eye movement. Bottom channel in *A* and *B* is the integrator record.

an artifactual deflection of the base line (arrow). Figure 10*B* shows the same insertion with eye movement in the opposite direction, again with an artifactual deflection of the base line (arrow), which may frequently be seen with a change of eye position. Figure 10*C* shows another marked artifactual shift in base line in another patient, which has often been mistaken for summated unit activity.

Figure 11*A* shows a saccadic movement *with* a burst of unit activity, and Figure 11*B*, the same *without* an initial burst of unit activity and *without* shifting of the base line.

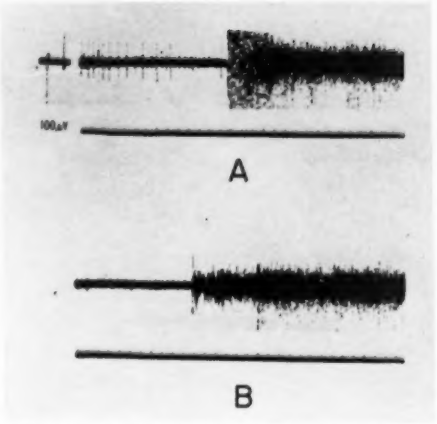


Fig. 11.—*A*, a saccadic movement with a burst of unit activity. *B*, without an initial burst of unit activity. Neither shows an artifactual shifting of base line.

Figure 12 shows a high-film-sweep-speed sample of a quick eye movement *without* an artifactual shifting of base line. Figure 7 shows a similar quick eye movement *with* a superimposed artifactual shifting of base line mixed with unit activity.

From the foregoing examples, it may be concluded that a proper evaluation of an ordinary sweep-speed electromyogram (½ in. per second) may only be made by a similar tracing of the same phenomenon with film sweep speed fast enough to exhibit the wave form of activity. The apparent burst may be a true increase of activity or may be solely an artifact or may be mixed, as will be discussed later.

The base-line shifts may be caused by the eyelids touching the electrode shaft. Movements of the lids against the electrode in an extraocular muscle, as during a blink, may produce characteristic recorded changes.

Figure 13*A* shows the effect of blinks on electromyographic tracings. Figure 13*B* shows a fast-film-sweep-speed presentation of a blink (arrow) superimposed upon normal unit activity. A blink is recorded as a shift of base line and may at slow film sweep speed simulate unit activity or a burst of unit activity.

3. *Orbicularis Muscle Activity.*—This may also be recorded as an artifact from an electrode in an oculorotary muscle. Gentle or strong voluntary closing of the eyelids results in marked increase of electrical activity from every extraocular muscle being recorded (Fig. 14).

Although this might at first observation appear to indicate coactivity of the extraocular muscles during lid closure, the following experiment shows that it is not the case.

When an electrode is inserted into the conjunctiva alone *over* a rectus muscle but *not into* the muscle (which position does not show unit activity with eye movements), then lid closure still yields marked activity in all channels (Fig. 15).

Lid hooks, which hold the lids free of the globe and away from the electrodes, result in a diminshed recording of activity noted during voluntary attempted lid closure and blinks (Fig. 16*A* and *B*). Presumably, there is a decrease of the transmission of orbicularis activity to the activity recording electrode inserted into the oculorotary muscle. Hence, it is important to remember that one may record electrical activity, presumably from the orbicularis, from electrodes inserted into the oculorotary muscles and that such extraneous electrical activity on the recording may not be satisfactorily elimi-

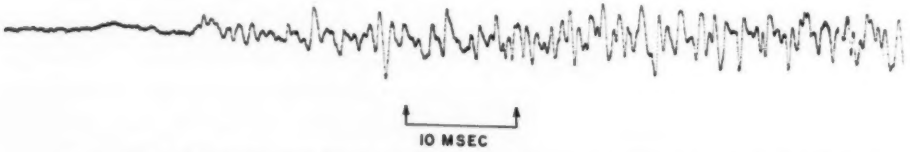


Fig. 12.—A high-film-sweep-speed sample of a quick eye movement without an artifactual shifting of base line.

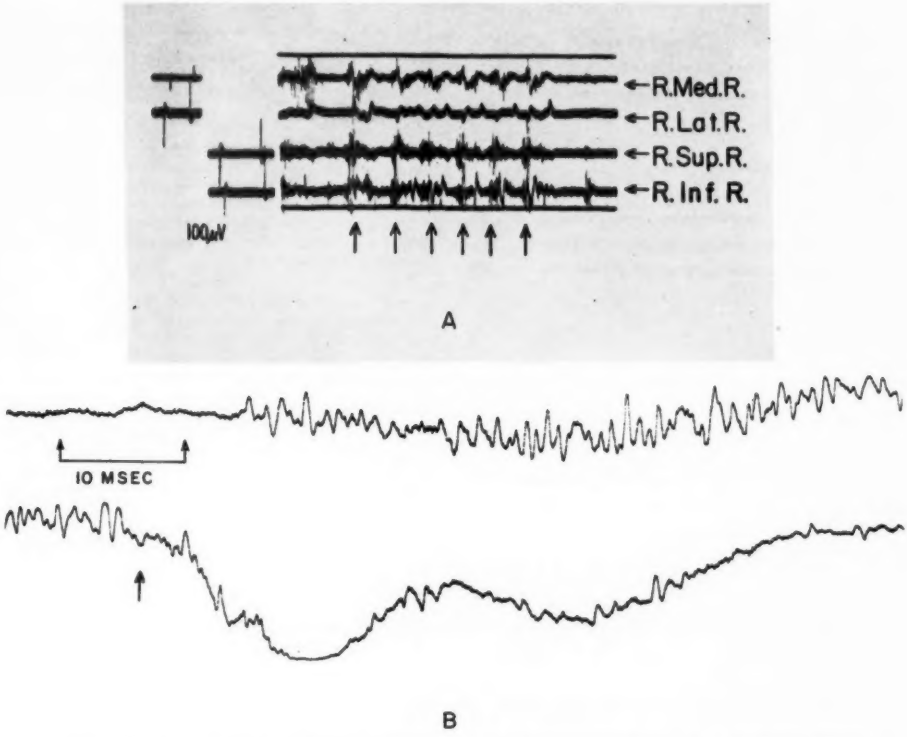


Fig. 13.—*A*, the effect of blinks (arrows) on the electromyograms. *B*, a fast-film-sweep-speed presentation of a blink superimposed upon normal unit activity. The lower trace is a continuation of the upper trace. Arrow indicates the start of the blink.

Fig. 14. — Voluntary closing of the eyelids, showing increased recorded electrical activity from all electrodes inserted into the rectus muscles. The lower tracing is the time signal and is the same for Figures 14-17, 20, and 21.

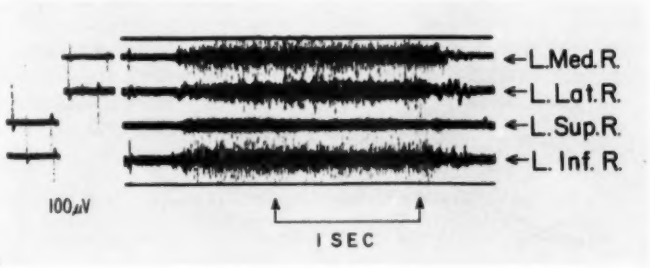
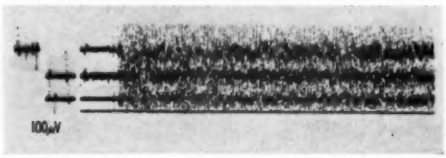


Fig. 15.—Voluntary closing of the eyelids, resulting in an increased electrical activity recorded from all electrodes inserted into the conjunctiva near, but not into, the rectus muscles.



nated by usual equipment alterations or usual shielding techniques.

4. *Artifacts Recorded with Poor Electrode Insertion or Low-Amplitude Recording.*—Figure 17 shows a poor electrode insertion (Channel 1) with consequent low amplitude of activity. It is noted that artifacts are more evident in records with poor

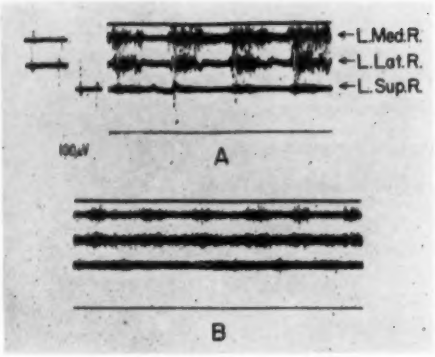


Figure 16

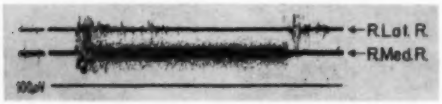


Figure 17

Fig. 16.—Voluntary closing of the eyelids and blinks under usual circumstances, *A*, showing bursts of increased recorded activity from electrodes inserted into the rectus muscles. *B*, same as *A* with lid hooks, which hold the lids free of the globe and away from the electrodes, resulting in diminished recorded activity.

Fig. 17.—A normal recording of saccadic movements from the right medial rectus and a poor recording with low amplitude of activity from the right lateral rectus, owing to poor electrode insertion. Note that artifactual activity is more evident in the tracing from the poor insertion.

electrode insertions and low amplitude of activity. These errors may be minimized or eliminated with good engineering and good insertions of electrodes into the muscles, so that recordings of sufficient amplitude are made. One must, therefore, be especially cautious in interpreting records from paretic muscles.

5. *Improper Shielding and Inadequate Amplifier Balance.*—These factors may allow recording of extraneous electrical activity, such as heart beat, nasopharyngeal muscular movement, and diathermy machine activity.

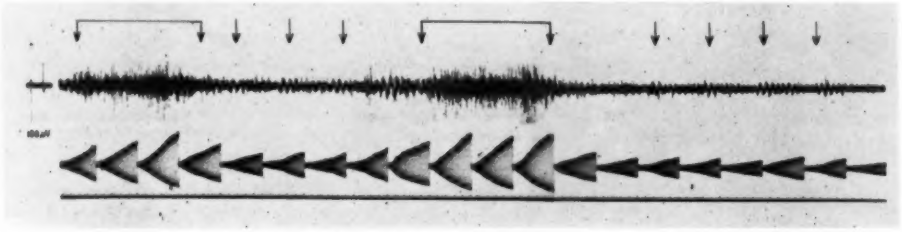
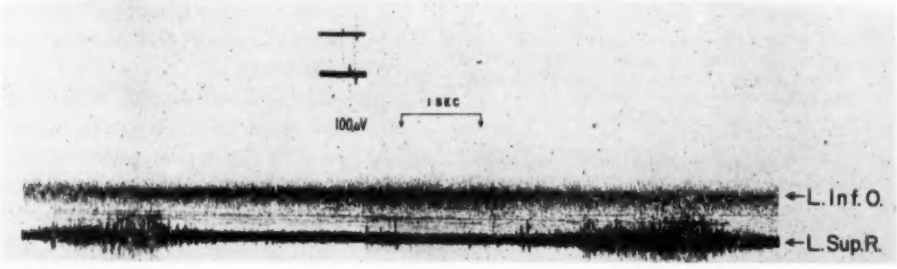


Fig. 18.—Rhythmic artifacts from the heart beat (arrows) and from swallowing movements (brackets) recorded from an electrode inserted into an extraocular muscle. Note that the integrator (Channel 2) reflects artifactual activity.

(*a*) Rhythmic Artifacts: Rhythmic artifacts, such as electrical activity resulting from the heart beat (Fig. 18, arrows) or from swallowing movements (Fig. 18, brackets), may be recorded from an electrode inserted into an extraocular muscle.

Fig. 19.—Waves of activity from a clinically involved extraocular muscle (left superior rectus) in a patient with endocrine exophthalmos during attempt to hold steady fixation in the field of its action. (Incidentally, note relatively unchanged activity of left inferior oblique, with, at the same time, waves of activity in left superior rectus.)



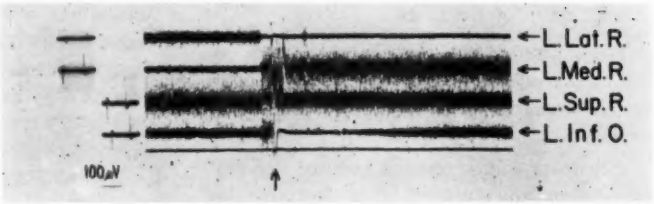


Fig. 20.—Blocking (beginning at arrow) of tracing from the left inferior oblique because of overloading of the amplifier.

This artifact may be eliminated by proper amplifier balance. Some of these rhythmic changes may be confused with waxing and waning of activity, so-called "spindle pattern" activity.

Incidentally, other waves of activity, which may be regular or irregular, may occur in patients with attempted gaze during fatigue and also in attempted gaze in anomalous conditions, such as muscle paresis and exophthalmos, when there is difficulty in sustaining fixation in a particular field of gaze. Figure 19 demonstrates waves of activity in the superior rectus during attempted sustained fixation in the upper field in a patient with endocrine exophthalmos. Similar phenomena of uneven recruitment were described earlier by Björk.[5]

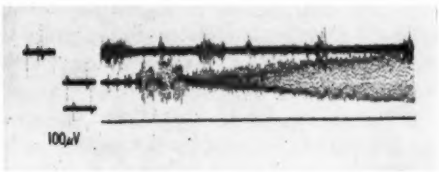


Fig. 21.—Simulated unit activity caused by oscillation of the amplifier.

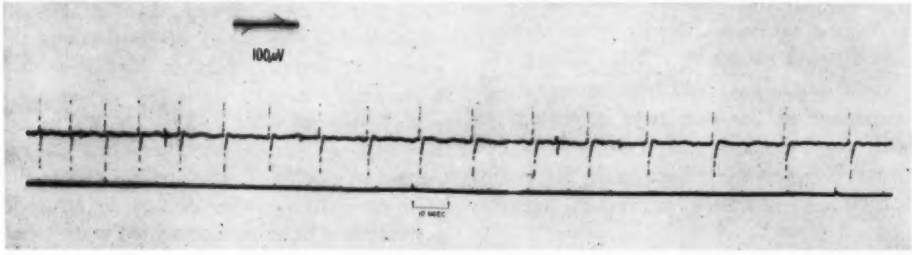
(*b*) Overloading of the Amplifier: This may cause blocking (Fig. 20, fourth channel, beginning at arrow).† The second channel in Figure 21 shows oscillation of the amplifier, an uncommon occurrence, which may simulate unit activity.

(*c*) Variations in Film Sweep Speed: Changes in the camera-film sweep speed during a given recording may cause an apparent change in frequency of single units. This is particularly true at the beginning or end of a record when the film may be accelerating or decelerating. Figure 22 shows single-unit activity during an acceleration phase of the film. One will note that as the camera-film speed increases, the unit-activity wave form becomes spread out from the straight line spikes at the beginning of the tracing and is clearly displayed.

Such alterations in camera-film sweep speed may be monitored by simultaneous recording of a timing signal or eliminated by the camera design.

† Amplifier overload results when an input signal, considerably larger than the amplifier was intended to handle, is applied at the input. Following transient overload, an A. C. amplifier is blocked for a period between a few hundredths of a second and several seconds. During the blocked period the amplifier does not work.

Fig. 22.—Single-unit activity during an acceleration phase of the camera film speed. Note apparent change in frequency of the unit activity and the corresponding change in the time-scale tracing.



Comment

There are many normal variations in recorded electrical activity from human ocular electromyography, and there are many artifacts.

Whenever a new concept is to be derived from electromyography, it is desirable to observe the following criteria: (*a*) a typical slow-film-sweep-speed recording of local electromyographic activity with adequate amplification; (*b*) a fast-film-sweep-speed recording of the same phenomenon, in order to examine the wave form and unit activity, and (*c*) information relative to the presence or absence of fusion and maintenance of fixation.

It is of especial importance to analyze a fast-film-sweep-speed sample in order to distinguish artifacts from true unit activity, not always distinguishable in recordings at slower speeds.

Whenever an eye moves, one may record artifacts of base-line movement. It is probable that artifacts are magnified in different eye positions and with increased unit activity.

Shifts of base line may be confused with muscle-unit activity, and this may lead to confusion in interpretation relative to time relationships of innervation, muscle contraction, and eye movement. Eye movement or position may *not* with any degree of certainty be extracted from an electromyogram. One may have electromyographic changes from the recorded muscle without eye movement, and one may, under certain conditions, occasionally have eye movement without electromyographic changes. Eye movement is better determined and measured by other techniques, such as electro-oculography.

A good electrode insertion with adequate amplification of the record is desirable for proper evaluation. Artifacts appear to be commoner in low-amplitude recordings or with poor electrode insertion, and here a fast-film-sweep-speed sample of the record is even more desirable for proper interpretation.

Before the following interpretations and concepts of electromyographic data become too firmly entrenched in the literature, it may be well to examine them more closely in the light of findings presented in this paper.

1. *"Spastic Esotropia."* [6]—One cannot determine relative strengths of activity from electromyograms except in marked paresis. Electrode-placement variation may account for large differences in amplitude of the recording. A large amplitude recording of the medial rectus muscle does not establish a diagnosis of "spastic esotropia."

2. *"Retraction Nystagmus."*—We agree with Björk [7] that "in the recording of nystagmus, artifacts easily occur, and may be due to movement of the needle electrode. Interpretation of the records must therefore be done with caution." Electromyographic evidence in support of "retraction nystagmus" could be due to artifacts originating in the eyelids. Simultaneous recording of the electrical artifacts on multichannel records makes their recognition easier.

3. *"Anomalous Innervation."* [8]—Some of the evidence in support of the phenomenon of "anomalous innervation" could, in part, be normal electromyographic variations. It has been pointed out that low-amplitude recordings, such as from paretic muscles, are particularly prone to artifacts. Recording through a single electrode the activity from *both* inferior oblique and inferior rectus muscles may be a normal variation and, thus, insufficient evidence in support of this concept. Base-line artifacts and other extraneous electrical phenomena recorded (such as television) may be systematically changed in amplitude with alterations in eye position and with changes in unit activity. It is therefore felt that the unusual physiological hypothesis of "anomalous innervation" requires further elucidation and more evidence.

4. *"Hering's Law."*—This law and its interpretation relative to alterations in recorded coactivity of opposing muscles in the stationary fixing eye during asymmetric convergence have been presented elsewhere.[9]

Slow eye movements of less than 8 degrees do not ordinarily show observable alterations in recorded electrical activity from the ocular muscles. Thus, significant relative changes in electromyograms may not be obtained because of insufficient instrument sensitivity (amplification) or insufficient stimulus. This has led to what we believe to be an error in the interpretation of Hering's law by other authors.

5. *"Divergence Mechanism."*—This mechanism is best studied electromyographically by simultaneous binocular recordings.

It is hazardous and, indeed, often erroneous to make inferences regarding *binocular* functions or mechanisms from *monocular* electromyograms.[10] *Monocular* electromyograms from an abducting eye, under whatever circumstances, indicate only that when an eye moves the movement is the result of oculorotary muscle contraction. The recorded electrical unit activity indicates the contraction, but not the function, of the muscle at any particular time. Thus, an electromyogram of the horizontal rectus muscles of one eye cannot be used to distinguish divergence from abduction.

We can understand Sir Francis Walshe's pessimism regarding electrophysiology, quoted in the opening paragraph, but we cannot agree with his conclusions. With strong skepticism and painstaking care, artifacts may be largely, if not entirely, exposed and eliminated or rejected.

Niles Roth, A.B., M.O., designed and constructed the electrode; William Houweling constructed, maintained, and operated the electronic equipment; Wilmer Renner took the photographs.

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Histochemistry of the Retina in Tay-Sachs Disease

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The pathologic features of Tay-Sachs disease have been described by numerous investigators.[1] All are agreed that the significant abnormality in the eye is a distention of the ganglion cells about the fovea and, with rare and inexplicable exception,[2] an atrophy of the optic nerve. Especially valuable from the histologic point of view have been the studies on eyes fixed within a matter of minutes after death.[2,3] The swollen appearance of the ganglion cells in eyes subjected to routine dehydration suggested early that the abnormal substance was nucleoproteid,[4] myelin,[3] or fat, but Marchi stains for fat were negative.[3] Subsequently, however, the substance was found to be sudanophilic[2,5-7] and presumably identical with the partially characterized substances found in the ganglion cells of the brain with this disease.[8-12]

Despite the number of well-documented studies with use of standard histologic methods, the retina of this disease has not been extensively investigated by means of modern histochemical techniques. It is the purpose of this article to report a study directed toward this end and to emphasize several features not heretofore recognized.

Report of a Case

This patient was a Jewish boy who had been followed by the Children's Medical Center of Boston and was at the time of death 3 $\frac{5}{12}$ years old. The other two siblings in the family were normal, but one paternal cousin had died of Tay-Sachs disease.

A diagnosis of Tay-Sachs disease was made in the present patient at the age of 6 months on the basis of cherry red foveas and convulsions. He was hospitalized several times for bronchopneumonia and put on several lipid-mobilizing agents (Monichol, Tween, choline, and inositol), as well as anticonvulsants. He became progressively listless and blind, however, and eventually he was completely out of contact with his environment. Finally he died at home from aspiration pneumonia and was transferred to the hospital for autopsy.

The eyes were removed and fixed in formalin for several weeks. The globes were then bisected, and the anterior and posterior halves were embedded and sectioned separately. The anterior halves were interpreted as normal.

The posterior half of one eye was embedded in gelatin and most of it cut in the frozen state. A small portion of this retina was excised, dehydrated, and embedded in paraffin. The optic nerve of the other eye was separately excised, embedded in paraffin, and prepared as cross sections. The retina of the latter eye was embedded in gelatin and cut so as to obtain flat or oblique sections of the retina through the macular region. Control eyes that were presumed to be normal were treated in the same way.

Tissue Methods

For purpose of comparison, the present case was prepared along with and in an identical manner as eight normal eyes, the eyes of two patients with metachromatic leukoencephalopathy, and one eye of a patient with spongy degeneration of the nervous system. The decision as to whether or not changes in the present case were abnormal is based on comparison with these controls.

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Howe Laboratory of Ophthalmology Harvard University Medical School, and Massachusetts Eye and Ear Infirmary.

For solubility tests, unstained sections were immersed in various solvents for different periods of time and then mounted in Paragon Mounting Medium and examined for persistence of the abnormal birefringence which had been found in the untreated sections.

Observations

Gross Specimen.—The only definite abnormality in the gross was the dense white opacity corresponding to the macula and a central dark spot corresponding to the fovea. The size of the white area was approximately twice that of the disc. The disc itself appeared white, but this is not abnormal for enucleated and fixed eyes.

Unstained Sections.—These were not noteworthy except for the abnormal birefringent material, which will be described subsequently.

Hematoxylin and Eosin Stain.—Hematoxylin and eosin stains of the paraffin-embedded retina showed loss of the nerve fiber layer, reduction of the ganglion cells (especially in the immediate vicinity of the fovea), and a lightly eosinophilic and amorphous material in the inner layers of the retina about the macula (Fig. 1). This substance appeared to have been derived from the ganglion cells but was accompanied by no phagocytic or other tissue reaction. The ganglion cells were probably distended, and the cytoplasm appeared unusually empty, but most conspicuously abnormal was the presence of cell "ghosts" that had no nuclei. The rest of the retina appeared normal.

Sudan IV and Hematoxylin.—The ganglion-cell layer about the fovea showed a diffuse sudanophilia (Fig. 2). This was in the form of granules approximately 1μ in diameter present within ganglion cells and in the extracellular substance about ganglion cells. It was also present in large cells in the inner aspect of the bipolar-cell layer. While the sudanophilia was less marked than that, for instance, in the retrobulbar adipose tissue, it was considerably more marked than the normal sudanophilia in the terminal portion of the rods and cones. The variation in intensity of the staining was a function of the concentration of the granules rather than of the density of stain of the individual granules. No sudanophilia was present in any of the normal eyes, but there was some sudanophilia, although mild, of the large ganglion cells of the retina in the cases of metachromatic leukoencephalopathy. In these latter cases, however, in contradistinction to the present case, the ganglion cells about the fovea were less affected than the more peripheral ganglion cells.

The optic nerve showed none of the sudanophilia such as is normal for myelinated nerves.

Also evident in these sections was the extensive atrophy of the nerve fiber layer and the increased cellularity (gliosis) of the nerve head.

Sudan Black.—These sections showed the same sudanophilia, but more strikingly, as Sudan IV. Especially well seen was the sudanophilia of the cells in the inner portion of the bipolar-cell layer, in addition to that of the ganglion-cell layer.

Periodic Acid-Schiff Reaction.—Of all the stains, that with the P. A. S. technique yielded particularly striking features (Fig. 3). The ganglion-cell layer about the fovea and the inner portion of the bipolar layer even at some distance from the fovea stained a dark red. In the ganglion-cell layer the material within the cells and outside of the cells stained darkly resulting in a lamina of dark red material beneath the internal limiting membrane. That in the inner portion of the bipolar-cell layer was within cells and involved approximately every third cell in the innermost margin. It was thus commoner than would be expected if it depended on amacrine cells. These cells appeared to be typical ganglion cells. This stainability was not diminished by prior incubation with saliva or with hyaluronidase. Nor was it affected by soaking the sections in acetone for as long as one hour. It was, however, slowly abolished by soaking in 100% ethyl alcohol (ethanol) and promptly abolished by immersion in chloroform-methanol.

Normally ganglion cells take only a faint stain with the periodic acid-Schiff technique, but in the cases of metachromatic leukoencephalopathy the large ganglion cells away from the fovea showed a definite stain (although not so intense as in the foveal ganglion cells in the present case).

The optic nerve showed only a faint coloration in contrast to the deep red which is normal for myelin.

Cresyl Violet.—The ganglion cells about the fovea and the inner portion of the bipolar-cell layer showed a striking metachromasia so long as the staining had been sufficiently intense (Fig. 4). With lighter staining the metachromasia was most marked in the extracellular substance and in disintegrating ganglion and bipolar cells (Fig. 5). The staining of the most nearly normal ganglion cells was with the light cresyl violet stain a pale blue. In other words, it appeared that the substance became metachromatic as the cells died or as the substance became extracellular.

Between the connective tissue bundles the optic nerve showed a metachromasia which, although less intense than normal, was nevertheless definite. (The normal metachromasia of the ciliary nerves in these sections served as a standard for comparison.)

Nile Blue.—The ganglion-cell layer about the fovea showed a faint take but not more than normal. There was no rose-color staining except by the extrabulbar fat.

Baker's Chromate-Hematein Stain.—The ganglion cells about the fovea showed a mild but nonetheless definite blackening (Fig. 6). This appeared to involve both the intracellular and extracellular components. The ganglion-cell layer of the normal eyes showed no stain with Baker's method. The optic nerve of the present case showed no take with the chromate-hematein stain, whereas the normal eyes showed a dense stain (Figs. 9 and 10).

Paragon Stain.—Paragon stain was used on the flat sections of the retina. An intensely red color was produced in the substance of the ganglion-cell layer about the fovea and in the large cells of the bipolar-cell layer (Fig. 7). No such staining occurred in the normal control, and no other stain yielded such an effective differentiation of the abnormal substance in the retina in this case.

Birefringence.—Observations on the birefringent material in the retina was especially noteworthy, since it was a striking feature and, to our knowledge, has not previously been studied in the retina with this disease. The observations were made with a Zeiss microscope, with use of Polaroids on sections mounted in Paragon Mounting Media.

There appeared to be three types of birefringence which for purposes of convenience we shall describe under the headings of (1) macular birefringence, (2) reticular birefringence, and (3) artifact lipid crystals. They were seen in unstained

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Photomicrographs are cross sections of inner portion of retina in the parafoveolar regions of the macula. Microscopic magnification was × 125.

Fig. 1.—Hematoxylin and eosin stain. Most of the ganglion cells have disappeared, leaving an eosinophilic residue which has caused no evident tissue reaction.

Fig. 2.—Sudan and hematoxylin stain. The material present in the inner layers of the retina is weakly but definitely sudanophilic.

Fig. 3.—Stained according to the periodic acid-Schiff procedure. Noteworthy is the dense stain of the inner layer of the retina and, to less extent, of the inner portion of the bipolar-cell layer.

Fig. 4.—Cresyl violet stain. Marked metachromasia of the inner layers of the retina.

Fig. 5.—Less intense stain with cresyl violet, showing preferential metachromasia of the extracellular components of the inner retinal layer.

Fig. 6.—Chromate-hematein stain of Baker, showing a positive take by the innermost layers of the retina.

Fig. 7.—Paragon stain. Abnormal cells in bipolar layer showing granular and vividly red cytoplasm.

Fig. 8.—Photograph taken between partially closed Polaroids, showing the conspicuous birefringence of the substance in the innermost layers of the retina.

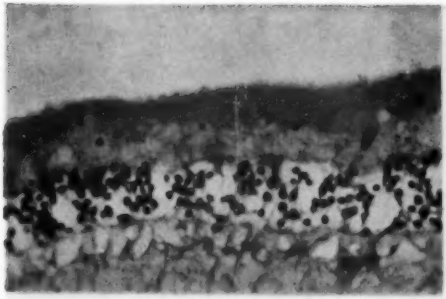


Figure 1

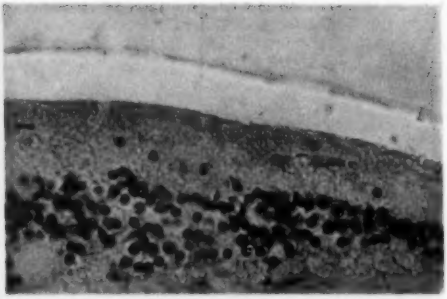
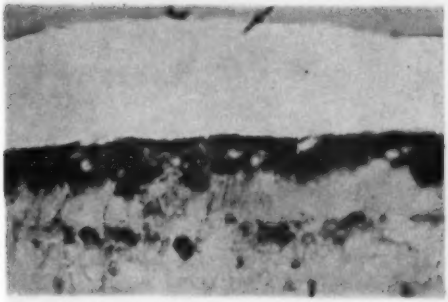


Figure 2



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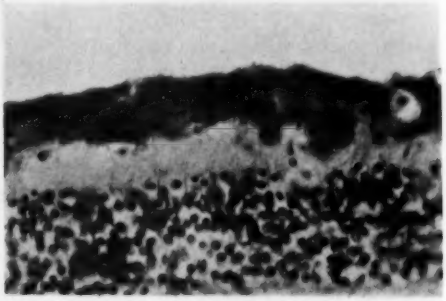


Figure 4

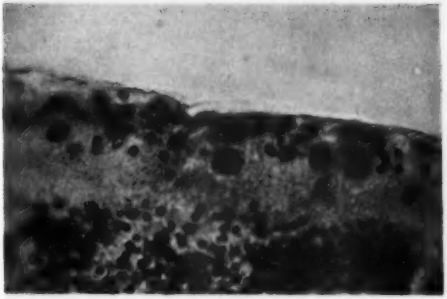


Figure 5

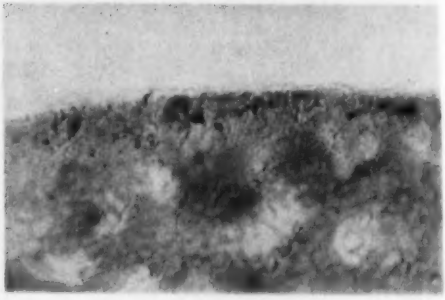
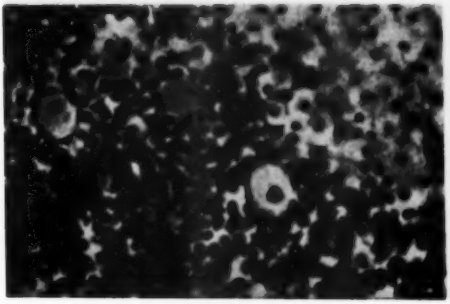


Figure 6



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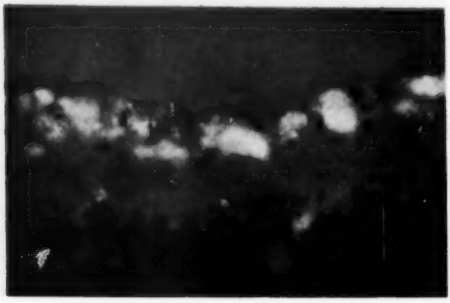

Figure 8

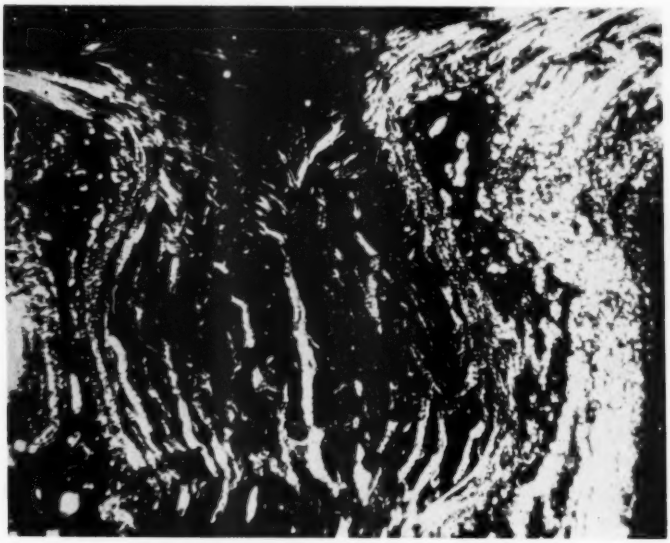

Fig. 9. — Longitudinal section of optic nerve at its entrance into the eye. Photograph taken with crossed Polaroids to show normal birefringence of collagenous framework but absence of the birefringence which is normally produced by myelin.

sections and in sections stained with cresyl violet and Nile blue. These stains did not involve the use of organic solvents.

1. Macular Birefringence: This was found most markedly in the inner portion of the ganglion-cell layer about the fovea abutting against the internal limbal membrane (Fig. 8). It had a characteristic granular configuration with an occasional Maltese cross. It was relatively brilliant, and, although white in unstained sections, it had a sky-blue color in sections lightly stained with cresyl violet. When viewed with partially crossed Polaroids in sections stained with basophilic dyes, it appeared to be chiefly extracellular. Intact ganglion cells showed relatively little birefringent material, and the impression was inescapable that the substance, originally found in the ganglion cells, became progressively birefringent with

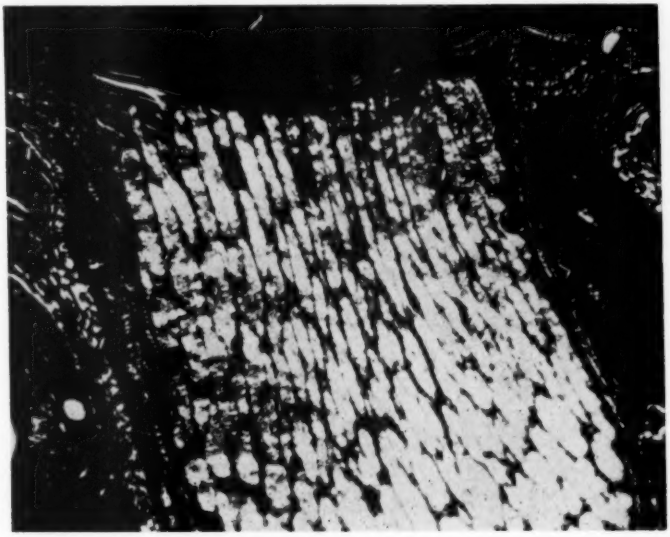

Fig. 10. — Comparable photograph of a normal optic nerve to show the birefringence which is normally produced by myelinated fibers.

disintegration of the cells. Nothing similar to this was found in the normal eyes or in the eyes from cases with metachromatic leukoencephalopathy or spongy degeneration of the brain.

2. Reticular Birefringence: Reticular birefringence is so called because it was found in the reticular layers of the retina between the nuclear layers throughout the retina. It is best observed on the nasal side of the disc, away from the macular birefringence. It is a smudge type of birefringence, without definite form, and is present to some extent in normal eyes. The reason for emphasizing it here is that it was considerably more marked in the present case than in any of the other eyes examined, but whether or not it has significance must await comparable observations in other cases of Tay-Sachs disease.

3. Artifact Lipid Crystals: These were present to a slight extent in this case as in many post-mortem eyes. They have no particular significance for the present case and are not described here further.

The optic nerve of the present case showed none of the granular birefringence which is normal for myelinated structures (Figs. 9 and 10). This was readily evident in frozen undehydrated sections and thereby provided a quick and easy method for the detection of optic atrophy. The linear birefringence of the collagenous framework, however, was preserved and possibly increased.

Baker's Chromate-Hematein Method.—This was negative for the optic nerve, while being strongly positive for the myelin of the ciliary nerves in the present case and for the optic nerve of control cases (Figs. 11 and 12).

Cross sections of the optic nerve showed the following:

Hematoxylin and Eosin.—The only abnormality noted was a two- to threefold increase in the cellularity of the interseptal areas, interpreted as gliosis, and possibly some increase in bulk of the septa. Except for these changes, the nerve appeared surprisingly normal.

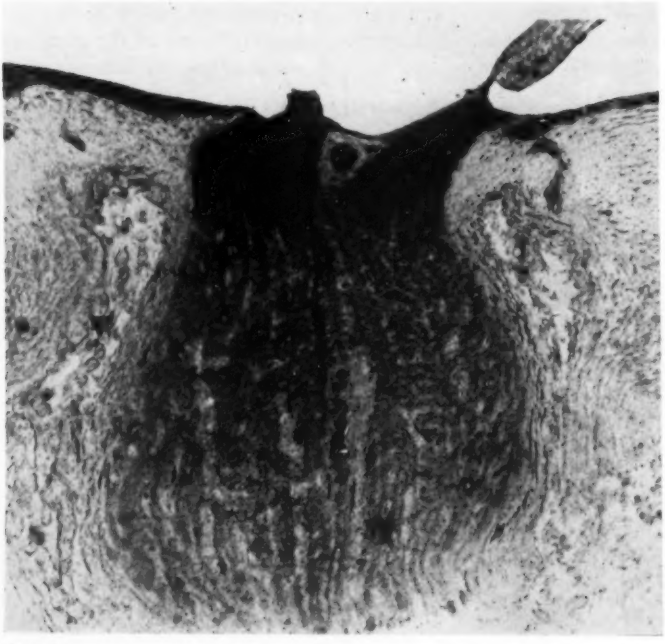

Fig. 11.—Baker's chromate-hematein stain of the optic nerve of the present case. There is no take by the optic nerve.

Fig. 12. — Comparable stain of a normal optic nerve. Myelinated structures show a strongly positive take.

Periodic Acid-Schiff Reaction.—The same changes were noted as found with hematoxylin and eosin.

Cajal.—No nerve filaments were demonstrated.

Davenport (Neurofibrillar Stain).—There were a few but greatly diminished number of neurofibrillae present.

Spielmeyer.—This was negative for myelin.

Holzer.—Mild gliosis.

Chloroform Methanol Solubility.—The insolubility of the foreign substance in acetone and its slow solubility in alcohol and rapid solubility in chloroform-methanol has already been noted in connection with the periodic acid-Schiff reaction. An easier test for presumptive solubility is the effect on the birefringent crystals with immersion in various solvents for different periods of time. By this means the substance was found to be insoluble in acetone, 0.1 N hydrochloric acid, and 0.1 N sodium hydroxide. On the other hand, it was slowly soluble in 100% alcohol and rapidly soluble in chloroform-methanol.

Comment

The foregoing findings confirm the observation of others on the sudanophilia of the parafoveal ganglion cells in this disease [2,5-7] and on the involvement of cells in the inner aspects of the bipolar-cell layer.[3] Moreover, the finding that the abnormal substance stains with the periodic acid-Schiff method and with Baker's chromatin-hematein method and is metachromatic is compatible with its being a glycolipid (possibly a phosphatide) with available acid groups as has been demonstrated by chemical analysis of the brain in cases of the Tay-Sachs form of amaurotic idiocy.[9-11] Its insolubility in acetone but solubility in other organic solvents is also compatible with such an identification. As has been suggested in the case of the retina [3] and of the brain,[9,12] the fact that this substance acquires the same tinctorial properties as myelin suggests an

anomalous process of myelin formation in cells that do not form myelin normally.

Aside from demonstrating a histochemical similarity of the substance in the retina with that which has been demonstrated chemically in the brain, the present case illustrates several features which have not been emphasized previously. First, the metachromasia occurs chiefly with disintegration of the cells. It is as though the acid groups of the polysaccharide became unbound as the cells died and thereby became sufficiently reactive to induce the metachromasia. Secondly, the abnormal substance in association with the ganglion-cell layer is strongly birefringent and may be readily visualized in the unstained state or when lightly stained with cresyl violet. It appears to arise in the ganglion cells but becomes deposited extracellularly. Nevertheless, even when extracellular, the substance gives rise to no phagocytosis or other tissue reaction.

The extensive involvement of cells in the inner portion of the bipolar-cell layer and of cells in the inner reticular layer was surprising to us, although it has been mentioned in previous publications. It would appear, however, from a casual study of normal infants' eyes that ganglion cells are much more abundant in these sites than is the case with adult eyes. This, if true, would account for the involvement of these areas.

Summary and Conclusions

The retina from a case of Tay-Sachs disease was studied with a variety of stains in an attempt to characterize the abnormal substance in the inner layers of the retina. Several eyes which were either normal or affected by some pathologic process other than that of Tay-Sachs disease were similarly stained and used for comparison. The following observations were considered noteworthy:

1. The abnormal substance in the retina is most conspicuous in the ganglion-cell layer but is also present to some extent in the inner portion of the bipolar-cell layer and the inner reticular layer. This is possibly attributable to the fact that ganglion cells are normally found in these sites, as well as in the ganglion-cell layer, in the infant's eye.

2. Arising apparently within the cells, the foreign substance eventually becomes largely extracellular, but, even so, it gives rise to no appreciable phagocytosis or other tissue reaction.

3. In accordance with current histochemical interpretations the substance fulfills the criteria for lipid (sudanophilia and solubility in organic solvents), vicinal groups (periodic acid-Schiff), and available acid groups (metachromasia). More specifically it is crystalline (birefringent) and gives the staining and solubility properties of being or containing a phospholipid (Baker's stain and acetone insolubility). The fact that the metachromasia becomes especially evident as the substance becomes extracellular suggests that the death of the cell is accompanied by release of the acid bonds.

4. The presence or absence of myelin birefringence in frozen undehydrated sections is presented as an easy way to detect optic atrophy.

243 Charles St. (14).

Addendum

Since this paper was submitted for publication, two papers have appeared reporting the histopathology of Tay-Sachs disease.[13,14]

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The Effect of Extraocular Muscle Contraction on the Elasticity of the Eye

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In previously reported work, an expression of the elastic properties of the adult human eye by means of the equation, E (coefficient of stretch) $= \frac{\Delta P}{\Delta V}$ (ΔP = pressure change, ΔV = volume change),[1] was proposed. When pressure change is determined per unit of time, the value of E for the mean of the pressure during this period can be obtained from prepared tables. With two of the variables known, i. e., E and ΔP in the equation $E = \frac{\Delta P}{\Delta V}$, the volume change ($\Delta V$) can be readily calculated. The applicability of this procedure for outflow measurements in the living eye awaits confirmation of the values of E.

It was felt that a study of the influence of some extraocular factors might promote a better understanding of the problem. The experiments of the present work deal with the effect of external pressure on the elastic properties of the cat eye. The Influence of various states of contraction of the extraocular muscles and that of diffusely exerted outside pressure on the globe were examined.

The relationship between ΔP and ΔV was studied in cats under three experimental conditions: first, before and after spastic contraction of extraocular muscles by decamethonium; second, before and during intracerebral electric stimulation of the third nerve, and finally, before and during rigor mortis. In addition, in one human eye the effect of rigor mortis on $\Delta P/\Delta V$ was determined.

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From the Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.

Methods

Young adult cats (1.8-2.5 kg.) were anesthetized with either pentobarbital sodium (35 mg. per kilogram, intraperitoneally) or chloralose (20 mg. per cubic centimeter of 70% ethyl alcohol, 2.0 cc. per kilogram, intraperitoneally). For post-mortem determinations the cats were killed by an overdose of pentobarbital sodium.

A tracheal cannula was inserted for artificial respiration, when required; the cannulated left femoral artery served for the continuous registration of the systemic blood pressure. Drugs and supplemental anesthetic were administered through a cannula in the left femoral vein. Intraocular pressure changes were measured by a Statham Transducer, Model P23BB, and changes of the systemic blood pressure by means of a Sanborn Electromanometer. Both functions were simultaneously recorded on a four-channel Sanborn Polygraph. Volume variations in the eye were induced by infusing fluid into the anterior chamber with the aid of a calibrated syringe microburet. The details of this method have been previously described.[2]

In four experiments, the pressure changes, produced by infusion of small fixed volumes of 0.9% solution of sodium chloride into the anterior chamber, were determined before the administration of decamethonium and after this agent, in a dose of 0.1 mg. per kilogram, had caused the extraocular striate muscle to contract. From one of these animals the experimental eye was removed at a time when the action of the

compound was still obvious, in order to repeat the elasticity determinations on the isolated globe.

The third nerve tract was electrically stimulated in four instances, with the cat fixed in a stereotaxic apparatus. The tip-to-tip distance of the bipolar electrodes measured 1 mm. For the location of the third nerve in the midbrain, use was made of the coordinates given in the atlas of Jasper and Ajmone-Marsan.[3] The responsive area was stimulated at a frequency of 60 cps, with a stimulus strength of 4 volts and a pulse duration of 2 milliseconds. The proper site of stimulation was indicated by sudden adduction and/or elevation of the globe, together with pupillary constriction on the ipsilateral side. Elasticity was determined before and during the electrically induced eye muscle contraction.

Measurements were obtained from four eyes of three cats four hours after death, when the animals showed signs of rigor mortis. The determinations were made first in situ and subsequently on the enucleated globes. Data obtained on one human eye in situ 30 minutes after death were compared with similar measurements carried out on another human eye after rigor mortis had developed.

The effect of diffuse external pressure exerted on the scleral coat was studied in four cat eyes by the following technique: The thumb finger of an autopsy glove served as a compartment to enclose the eye after a circular hole was cut at the tip of the finger. The edge of this hole fitted snugly against the limbus so that the whole cornea was exposed in the aperture. The rubber chamber was then filled with 0.9% solution of sodium chloride, and the palmar end of the finger was closed securely with a string. At some distance from the eye a hypodermic needle, connected with a saline reservoir, was introduced into the rubber chamber. By elevation of the reservoir, the pressure exerted on the scleral surface could be varied at will. Two No. 27 gauge needles were then inserted into the anterior chamber of the eye in order to infuse small quantities of fluid and to record the eye pressure. The elasticity of the eye was determined with pressures in the rubber chamber set at 0 and at 18 to 22 mm. Hg.

Results

Effect of Decamethonium Br+.—Figure 1*A* illustrates the relation of $\triangle P$ ($P_2 - P_1$) to $\triangle V$ of the cat eye measured before the administration of decamethonium bromide and when two doses of the compound (0.1 mg. per kilogram) had effected the maximal rise of the intraocular pressure. Also shown in this Figure is the response pattern of this eye immediately after enucleation. It resembles closely the type of reactions observed in three other experiments of this series. The elastic behavior of the eyes obtained prior to the injection of the pharmacological agent was similar to that previously reported.[2] With infusions of constant volumes of fluid $\triangle P$ progressively increased as the intraocular pressure rose. Above an eye pressure level of approximately 30-35 mm. Hg. $\triangle P$ became essentially constant.

The effect of the intravenous injection of 0.1 mg. per kilogram of decamethonium bromide on the elasticity was determined within one minute after its administration, while the animal was being given artificial respiration. The contraction of the extraocular muscles and the concomitant intraocular pressure rise (Fig. 2) lasted, generally, longer than 20 minutes. The responses of the eye to the infusion of 5 or 10 cu. mm. of fluid consisted of changes in $\triangle P$ smaller than those obtained before the injection of decamethonium bromide, becoming progressively larger as the intraocular pressure was increased. The control and experimental curves of $\triangle P/\triangle V$ for each eye could be superimposed, with good fit, by a shift of the absolute pressure scale (Fig. 1*B*). One of the five eyes of this group was enucleated while the effects of decamethonium bromide were still evident, as indicated by the elevated pressure in the contralateral eye. The elasticity was deter-

mined immediately after removal of the eye to ascertain whether decamethonium bromide had exerted some effect on the eye other than that mediated by the contraction of the extraocular muscles. The resulting curve of $\triangle P/\triangle V$ appears to be identical with the tracings obtained prior to drug administration.

Effect of Extraocular Striate Muscle Action Induced by Third Nerve Stimulation.—$\triangle P/\triangle V$ was measured in the four eyes of this series before and during intracranial

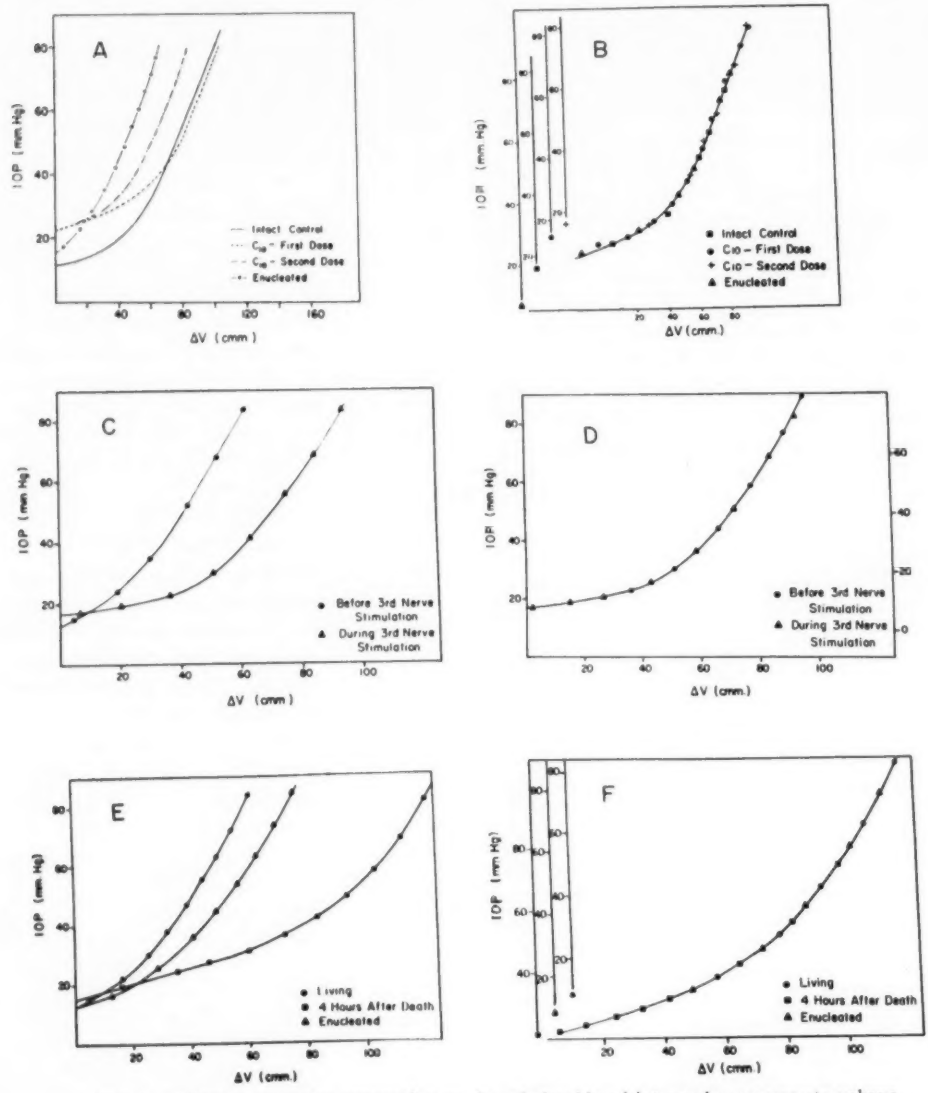
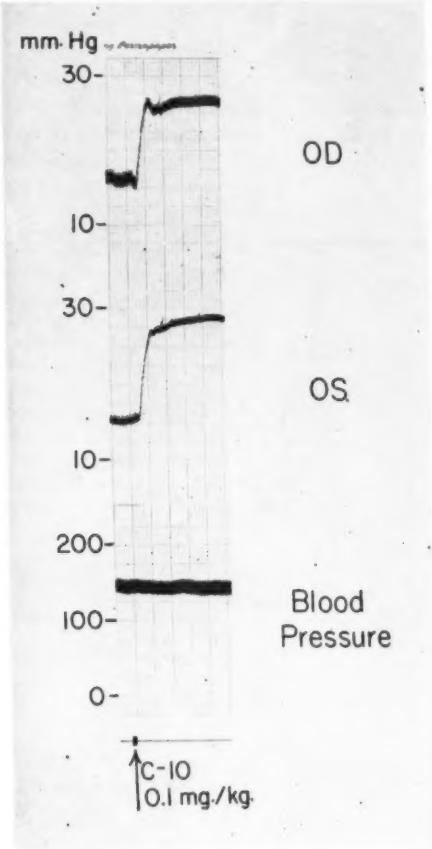


Fig. 1.—Effect of muscular contraction on the relationship of intraocular pressure to volume increment. On the left are plots of volume changes ($\triangle V$) versus absolute intraocular pressures (IOP). On the right, shifts of the absolute pressure scale, as indicated in the ordinates, permit the curves to be superimposed with a reasonably good fit. *A* and *B*, muscular contraction induced by decamethonium Br^+. *C* and *D*, muscular contraction induced by third nerve stimulation. *E* and *F*, muscular contraction during rigor mortis.

stimulation of the ocular motor nerve. Nerve stimulation resulted in an increase of intraocular pressure (Fig. 3). A decrease of $\triangle P$ was noted when comparisons were made with values determined before nerve stimulation at similar pressure levels (Fig. 1*C*). As the intraocular tension increased, $\triangle P$ became progressively greater. At pressures above 45-55 mm. Hg, $\triangle P$ remained fairly constant. The curves of $\triangle P/\triangle V$ for each eye, obtained before and during stimulation, could be easily superimposed with a good fit by shifting the absolute eye-pressure scale (Fig. 1*D*).

Fig. 2.—Effect of decamethonium Br^+ on the intraocular pressure. The intravenous administration of decamethonium Br^+ produces a rise of both eye pressures (O. S. and O. D.). No effect is seen on the blood pressure. Time: 10 seconds between heavy vertical bars.



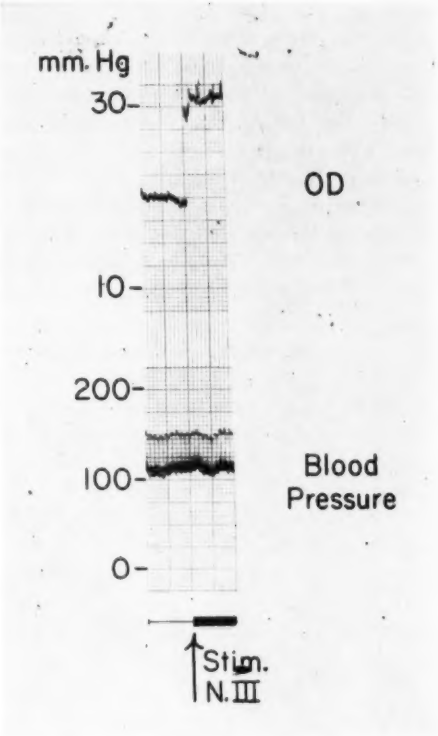


Fig. 3.—Effect of third nerve stimulation on the intraocular pressure. Intracranial stimulation of the third nerve produces movements of the eye and an elevation of eye pressure (upper tracing). No effect is seen on the blood pressure. Time: 10 seconds between heavy vertical bars.

Effect of Extraocular Striate Muscles in Rigor Mortis.—In this series of experiments, the contraction of extraocular striate muscles caused by rigor mortis produced changes which deviated from the elasticity patterns of controls in a manner similar to the changes which followed injection of decamethonium and which occurred during the third nerve stimulation, as shown in Figure 1*E*. In these cats, the patterns were determined in situ before the animal was killed and four hours post mortem; the procedure was repeated immediately after the eyes were enucleated. As in the preceding series, the curves obtained before and during muscle contraction caused by rigor mortis and those obtained after enucleation were superimposable with good

fit (Fig. 1*F*). One eye of a 7-year-old patient was examined in situ four hours after death, when signs of rigor mortis had developed. Enucleation was not permitted. The results were compared, therefore, with those obtained from another human eye (age of patient, 23 years) which was studied in situ 30 minutes post mortem (Fig. 4*A*). Under this condition of comparison, the eye tested four hours after death differed from the eye examined at the 30-minute interval to an extent almost identical to that observed in cat eyes. Again, the two elasticity curves could be superimposed with a reasonably good fit, as illustrated in Fig. 4*B*.

The Effect of Diffuse External Pressure. The compartmental pressure of approximately 20 mm. Hg exerted on the scleral coat caused a change in the $\triangle P/\triangle V$ relationship when compared to the same eyes at 0 mm. Hg pressure (Fig. 5). The change

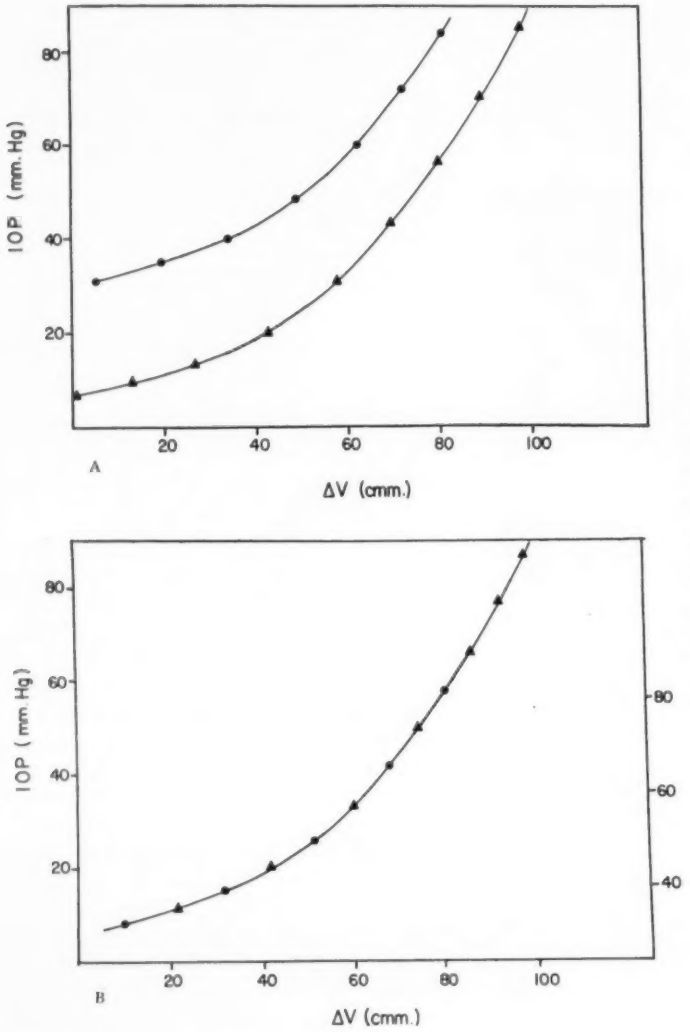


Fig. 4.—Effect of rigor mortis on the elasticity of the human eye. Circles indicate the data obtained from one human eye during rigor mortis. Triangles indicate similar data from another human eye obtained 30 minutes after death. *A*, $\triangle P$ plotted versus $\triangle V$ on an absolute pressure scale. *B*, superposition of both curves by a shift in the absolute pressure scale of the eye under rigor mortis, as indicated in the right ordinate.

was similar and in the same direction as that found with intact eyes under the influence of muscle spasm. The two curves could be superimposed by a shift of the absolute pressure scale equivalent to the compartmental pressure.

Comment

The similarity of the results obtained by three experimental conditions, namely, intravenous injection of decamethonium, third nerve stimulation, and rigor mortis, strengthened the concept that no mechanism other than the muscle contraction produced the elasticity changes.

The influence of decamethonium on the elasticity of the eye in situ was not observed when, after enucleation, the effect of muscular contraction was eliminated. Intracranial third nerve stimulation was followed by an immediate fast eye-pressure rise, which was attributed to the increase of tension of the responding oculomotor muscles. With the employed experimental technique, no other stimulation effect on either the elasticity or the intraocular pressure could be demonstrated.

In rigor mortis, the increase of striate muscle tension was also the cause of the change of the elasticity pattern. This was confirmed by the abolition of this phenomenon in the enucleated eye.

An increase of the muscle tension is the common factor in the induction of similar elasticity changes effected by three different procedures. It is concluded, therefore, that in the three experimental approaches, the muscular factor alone influenced the elastic behavior of the eyes.

When the tension of the striate eye muscles was increased under each of the three conditions, the slopes of $\triangle P/\triangle V$ were decreased when compared with the responses in control eyes at similar intraocular-pressure levels. This relationship was limited to a pressure range below the point at which $\triangle P/\triangle V$ became relatively constant under the influence of muscle spasm. Above this pressure value the difference between the experimental and the control series disappeared.

Two explanations for the observed effects of muscular contraction on the elasticity of the eye have been considered:

1. *Dimensional Change of the Eye.*—The elasticity as measured in these experiments is dependent upon the degree of stretch

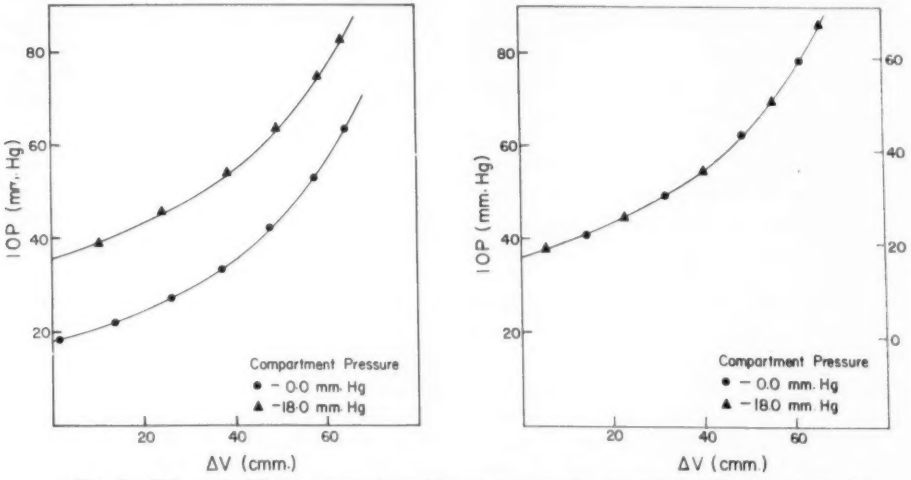


Fig. 5.—Effect of diffusely exerted outside pressure on the elasticity of the eye. Left, the pressure-volume increment relationship at compartment pressure of zero (circles) and 18mm. Hg (triangles). Right, the two curves are superimposable by an 18 mm. Hg shift of the absolute pressure scales as indicated at the ordinate. Left ordinate, scale for triangles; right ordinate scale for circles.

and, consequently, upon the area of the corneal-scleral coat at the time of the determinations. With a minimal stretch on the eye, $\triangle P/\triangle V$ would be small but would increase as the degree of distention became greater. In order to explain the decrease of the $\triangle P/\triangle V$ relationship induced by muscular contraction on this basis, it would be necessary to presume that the eye approaches the geometry of a perfect sphere. Under this condition, the eye would become more relaxed and the surface area decreased. Change of geometry in this manner, however, would produce a fall of internal pressure; such an explanation is not compatible with the experimental data. In all cases the contraction of the extrinsic eye muscles produced a rise of intraocular tension.

2. *Diffusely Exerted Outside Pressure.*—If the elevations of intraocular pressure were due to a diffuse outside force exerted on the eye by muscular contraction, the concomitant decrease of the elasticity becomes explainable. The contents of the eye can be considered essentially as being incompressible. When an outside force is exerted on the coats, no appreciable decrease of volume would be anticipated and thus the area would remain unchanged; however, the intraocular pressure would become elevated. With volume and area constant, no additional stretch would be exerted. Thus it may be postulated that the intraocular pressure could be raised by an outside force and still retain the elastic constants found for that eye before the pressure was affected. This reasoning is supported by the experimental data. The elasticity curves ($\triangle P/\triangle V$) reflecting the influence of muscle spasm changed as the intraocular pressure was increased in a manner similar to that obtained for the appropriate controls. They differed only in their absolute pressure values. The elasticity curves obtained without and during muscular contraction could be easily superimposed by a shift of the absolute pressure scale. The difference of the absolute pressure scales was found to be equal to the initial elevation of intraocular pressure produced by the muscle-contracting stimulus. That this interpretation is valid was demonstrated by studies on enucleated eyes. A rubber compartment was constructed so that an eye could be placed within it with the cornea exposed to the atmosphere in order to approximate as closely as possible the experimental conditions. An elevation of the compartment pressure of approximately 20 mm. Hg. caused an identical increase of the intraocular pressure. The elasticity determinations performed at 0 and increased compartment pressures were found to be identical if a value equivalent to the compartment pressure was subtracted from the absolute pressure of the determination made at the elevated level.

Summary and Conclusions

An increase of extraocular muscle tension produces an elevation of intraocular pressure and a change in the elasticity of the globe. When the elasticity of the eye is compared, on an absolute intraocular pressure basis, before and after muscle spasm, a definite change of this factor is noted. It is represented by an increase of distensibility of the eye after the extraocular striate muscles have contracted. However, if a correction is made by subtracting the intraocular pressure rise from the absolute intraocular pressure, as effected by muscular contraction, then the elasticity of the eye before and after the increase of muscle tension becomes the same.

Mr. J. G. Brown gave technical assistance.

Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health (14).

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Incision and Closure in Cataract Extraction

HAROLD G. SCHEIE, M.D., Philadelphia

I consider it a great honor to have been invited to give the Charles H. May Memorial Lecture. Dr. May was an accomplished ophthalmologist, teacher, and writer. Although I did not know him personally, I have a feeling of close acquaintance because of his widely known textbook. This book served as my introduction to ophthalmology and helped to create an interest which eventually led to my choice of ophthalmology as a career.

The invitation to give this lecture included a request to speak upon some phase of cataract extraction. I have chosen to discuss methods of incision and wound closure. These topics are two of the most important phases of cataract operation, and, although it has been over 200 years since Daviel first removed a lens, methods of making and closing the incision still are widely debated. I became extremely interested in methods of wound closure early in my residency, and, as a result of this interest, I have collected most of the literature on the subject. I have also had illustrations made of many of these closures, some of which will be shown tonight.

In this presentation certain basic types of wound closure will be discussed and an attempt will be made to evaluate each from the standpoint of the ideal, which, I might add, has not yet been described. Advantageous features of each will be indicated. Finally, I will describe briefly my own current technique and accredit the source from which this or that maneuver was derived, because my present method represents an attempt to utilize, in a practical way, as many as possible of the favorable features of previously described methods of wound closure. My present wound closure technique, therefore, is a composite of many persons' ideas. The incision may be somewhat original.

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From the Departments of Ophthalmology, Hospital of the University of Pennsylvania, and The Children's Hospital of Philadelphia.

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Review of Standard Techniques of Wound Closure

This presentation is not meant to be a complete review, and for this reason the reader is referred to the excellent article by Roper.[1] Numerous articles on wound closure continue to appear in the literature, but certain very basic principles are involved. Wound closure can be classified according to the tissues approximated: (1) conjunctival, (2) corneal, (3) corneoscleral, (4) scleral, and (5) sclerоconjunctival.

1. Conjunctival Wound Closures.—The first attempt at wound closure was that of Desmarres,[2] who in 1858 employed a conjunctival bridge which was allowed to remain uncut as the knife was brought upward (Fig. 1). Numerous types of conjunctival bridges were subsequently described, but none have been truly satisfactory. This also is true of all closures based upon conjunctival sutures. These methods include leaving a small conjunctival flap attached to the corneal flap while making the section, the Kuhnt flap (Fig. 1),[3] and the Van Lint flap (Fig. 1).[4]

Evaluation of Conjunctival Closures: McLean (1940),[5] in his classic article on wound closure, established certain important criteria by which the adequacy of wound closure can be judged. He stated that ideal sutures for cataract extraction must (1) provide firm closure; (2) be placed prior

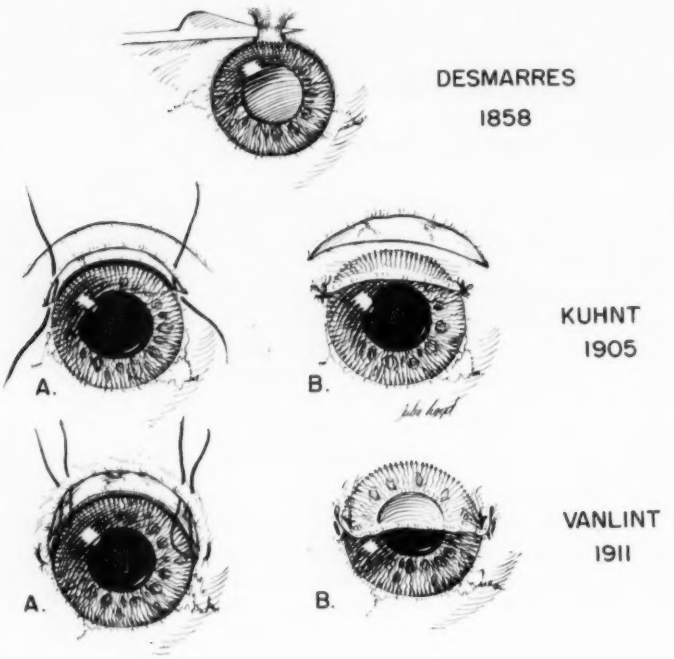


Fig. 1. — Conjunctival closures.

to section; (3) be inserted through, not over, the lips of the incision; (4) furnish a conjunctival flap to cover the incision, and (5) not be too complicated.

These criteria should be graded according to their importance to permit accurate evaluation of any single wound closure. Rated equally and of first importance should be firm closure and the placement of sutures through and not over the lips of the incision for purposes of good apposition. Next in order is an adequate conjunctival flap to reinforce and cover the incision. A conjunctival flap helps to prevent epithelial downgrowth, lowers the incidence of incarceration of the iris, and helps to prevent infection. Placement of protective sutures prior to the section is of somewhat secondary importance today because of our excellent anesthesia, effective techniques for obtaining almost uniform facial blocks, and excellent preoperative medication in the form of sedatives and tranquilizers. Preplacement, however, does lead theoretically to greater accuracy of wound closure. With present-day needles and suture materials the matter of a wound closure being too complicated is relatively unimportant. While it is true that some methods are technically more difficult and time-consuming than others, none of the methods thus far devised are too difficult for routine use by the average operator. The wound closure considered best by each individual ophthalmic surgeon should be employed, even though a few minutes might be required to complete the operation.

The various types of conjunctival closure fail to satisfy the two most important criteria listed by McLean, because they provide neither firm closure nor the good apposition that sutures through the lips of the incision can give. They are simple to use; some give an adequate conjunctival flap, and they can be placed prior to the section.

II. Corneal Sutures.—1. Williams (1865). The first firm wound closure described in the literature was the corneal suture devised by Williams (Fig. 2).[6] It was a single

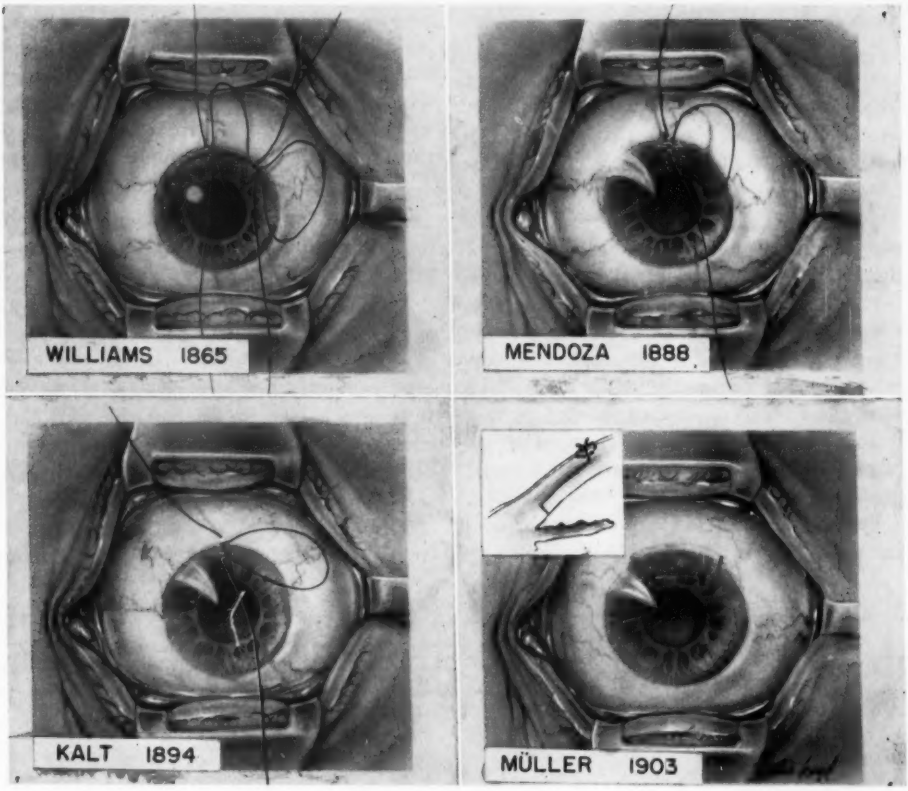


Fig. 2.—Corneal closures.

suture placed superficially through the cut edges of a corneal incision. Incisions at that time could be small and were usually corneal because the extracapsular technique was in vogue. Williams' method became popular, and the principle continues to be in wide use today.

2. Mendoza (1888). Suarez de Mendoza[7] introduced the principle of the preplaced suture (Fig. 2). He prepared a corneal gutter by incising partially through the thickness of the cornea without entering the anterior chamber. He then placed a suture through the cut edges and across the gutter, pulled the suture up to form a loop, and made a corneal incision between the arms of the loop. This principle has been used with many variations and forms the basis for many types of wound closure employed today.

3. Kalt (1894). Kalt[8] devised a bridge suture (Fig. 2). A bite was taken in the cornea about 1 mm. within and perpendicular to the limbus at the 12 o'clock position. The suture then was introduced in a similar way through the sclera directly above the limbus. In 1910 he modified his suture by placing the scleral bite horizontally. Although one arm of the suture is placed in cornea and one in sclera, the method was originally introduced to close a corneal section and, therefore, was truly a corneal suture. This method continues to be used by many ophthalmic surgeons.

4. Mueller (1903). Mueller[9] prepared a corneal flap by dissecting a superficial layer of the cornea upward toward the limbus (Fig. 2). Sutures were placed through the cut edges, and the section was

made beneath the corneal flap. The method has never become widely popular.

Evaluation of Corneal Sutures: When these basic corneal sutures are evaluated by the criteria of McLean, it is found that all give firm wound closure, which is a great advantage over the conjunctival closures. The Mendoza and Mueller sutures were preplaced and passed through the cut edges of the wound. As a result, they gave near-perfect apposition. The Williams suture, which was postplaced, gave less-perfect apposition. The Kalt method gave the poorest apposition, because it was a bridge-type suture, the arms passing over and not through the incision. All were simple to place, but the Mueller closure, because of dissection of a corneal flap, was the most difficult. The Kalt method was the easiest and safest. All of these closures failed to provide a conjunctival flap. Corneal closures are of great importance because they established most of the basic principles which are observed in the best present-day wound closures: (1) firm closure, (2) sutures through the lips of the incision, and (3) preplacement of sutures.

When corneal closures are evaluated by the criteria of McLean, it is found that all give firm wound closures.

III. Corneoscleral Sutures.—The problem of wound closure became even more urgent with the introduction of the intracapsular extraction and its popularization since the beginning of the century. This technique necessitated a larger opening for delivery of the intact lens, which, in turn, led to an increase of wound complications and a higher incidence of vitreous loss. The large incision was accomplished by making

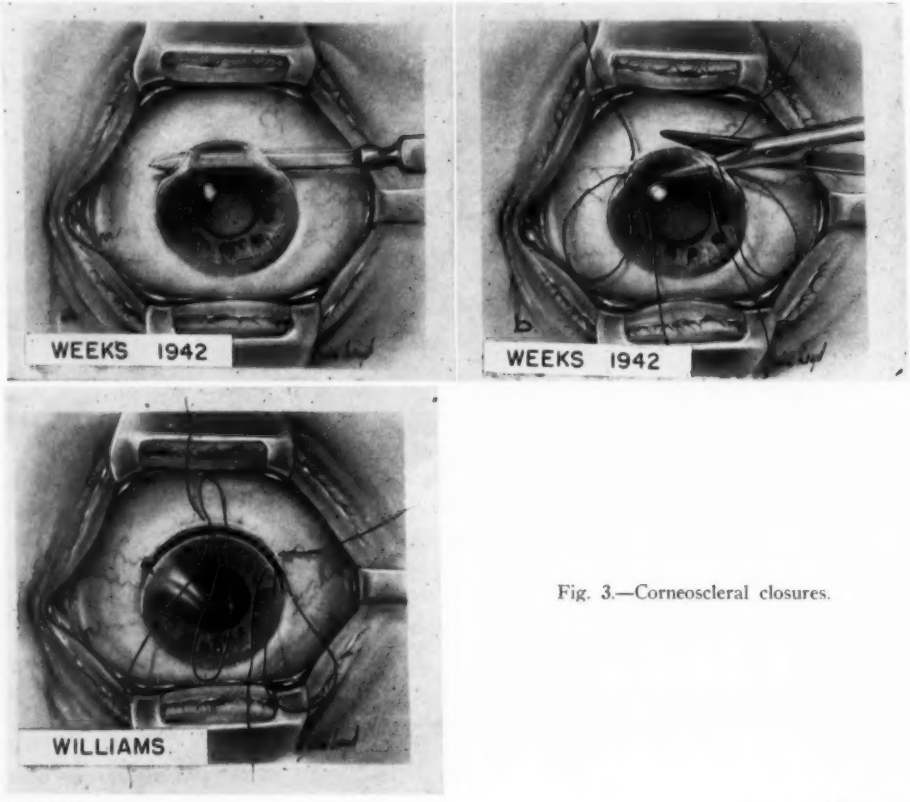


Fig. 3.—Corneoscleral closures.

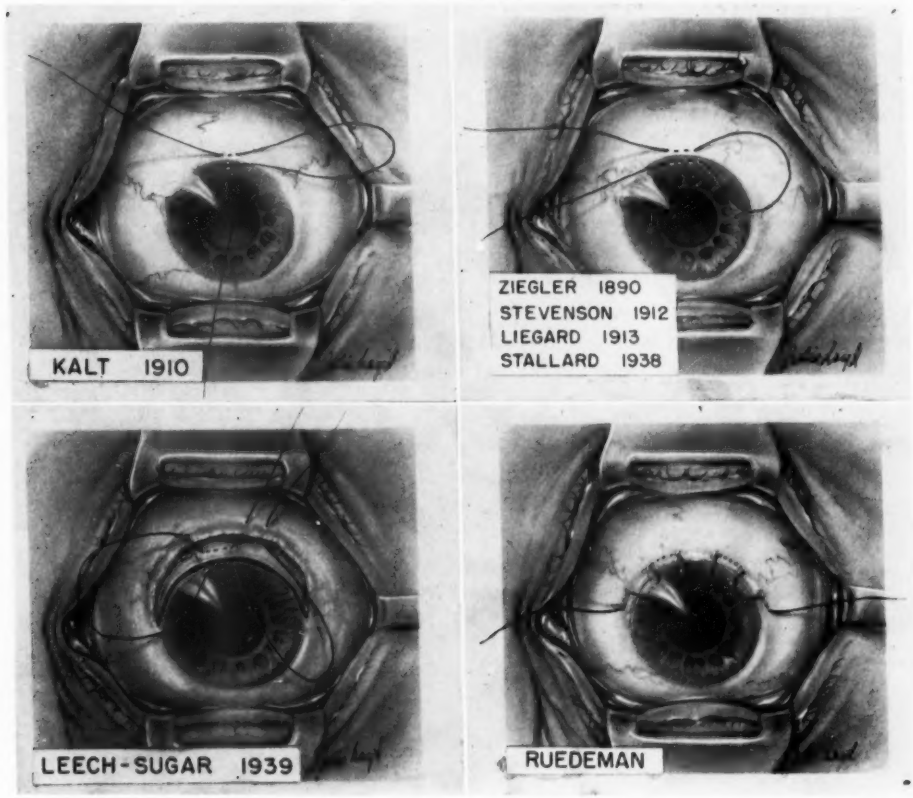
a full half-section and by placing the section at the limbus rather than within the cornea. Numerous techniques for closure of the corneoscleral incision were devised, but most of them embody modifications and refinements of the principles established by Williams or Mendoza. One of the most widely used modifications was the addition of a conjunctival flap.

1. Williams (1865). The Williams [6] type of suture, placed through the lips of a corneoscleral incision, has been among the most popular of all wound closures and is still used by a large number of ophthalmic surgeons (Fig. 3). It gave firm apposition and had the virtue of simplicity. This is particularly true today, with superb needles now available that can be inserted with relative safety even after completion of the incision.

2. Weeks (1942). Weeks [10] modified the Williams suture to overcome, at least partially, the objection that it was not preplaced (Fig. 3). He made an incomplete section with a Graefe knife, leaving an uncut bridge of corneoscleral tissue superiorly. Sutures were then placed near the upper extremities of the incision, nasally and temporally, adjacent to the uncut bridge. The sutures being near the ends of the incision, fairly great accuracy was assured in placing them. The section was completed with a scissors.

3. Kalt (1894). When the section was moved back to the limbus, the Kalt [8] suture became corneoscleral (Fig. 4). In 1910 Kalt modified his suture by placing the scleral bite horizontally. His method is still used by many surgeons, but it has undergone further modifications. Ziegler,[11] Steven-

Fig. 4.—Corneoscleral closures.

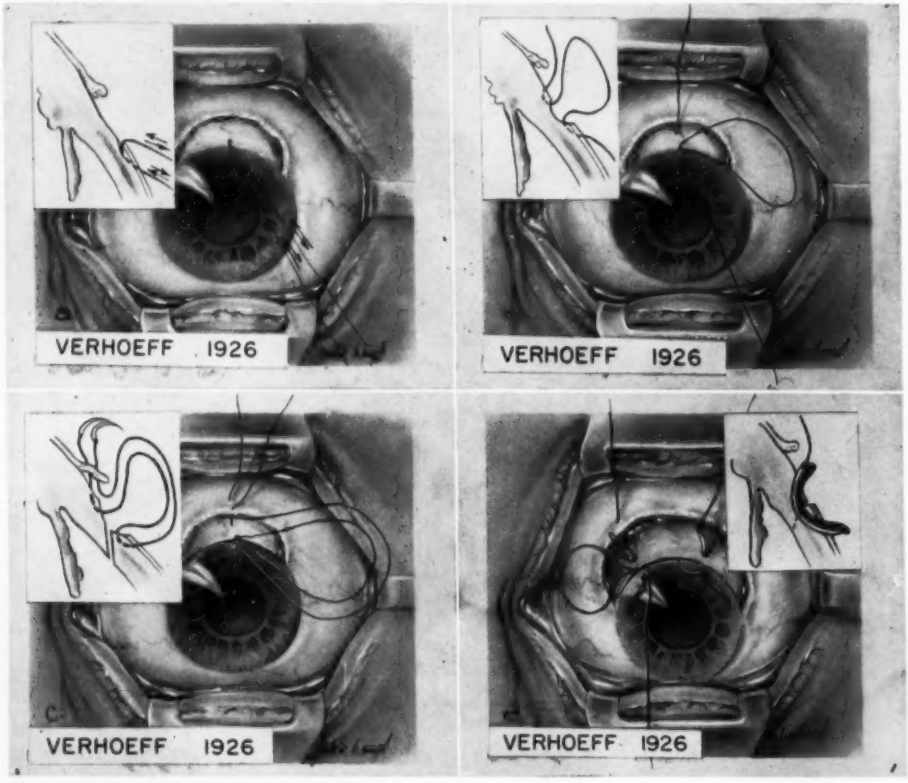


son,[12] Liegard,[13] and Stallard [14] placed the suture horizontally through both the cornea and the sclera. Some operators use more than one suture. Olmos [15] and Ruedemann [16] have used a continuous bridge suture (Fig. 4).

4. Leech-Sugar (1939). Leech and Sugar [17] modified the Kalt suture by preparing a fornix-based conjunctival flap (Fig. 4). Both arms of the Kalt suture were passed through the free edge of the conjunctival flap from the deep surface. When it was tied, the conjunctiva was pulled down over the incision.

5. Verhoeff (1926). Verhoeff [18] originated an excellent track suture which has been adopted by many surgeons throughout the world (Fig. 5). The conjunctiva was incised superiorly at the limbus, and a fornix-based flap was prepared. One or more sutures were inserted across the limbus, entering within clear cornea and emerging in sclera above. Each arm was cut to leave a few millimeters of suture protruding. A limbal incision was made which divided the suture where it was imbedded within corneoscleral tissue, one being retained in the corneal flap and the other in the sclera to provide markers along which a suture was threaded to close the wound. Both arms of this suture were passed through the conjunctival flap from its deep side to provide a cover for the incision when the suture was tied. Two other methods of inserting track sutures were described. One was done by staining the track with dye. The other was accomplished by inserting a suture vertically through the cornea, about 2 mm. below the limbus, emerging at the limbus. Both ends of the suture were

Fig. 5.—Verhoeff corneoscleral closure.



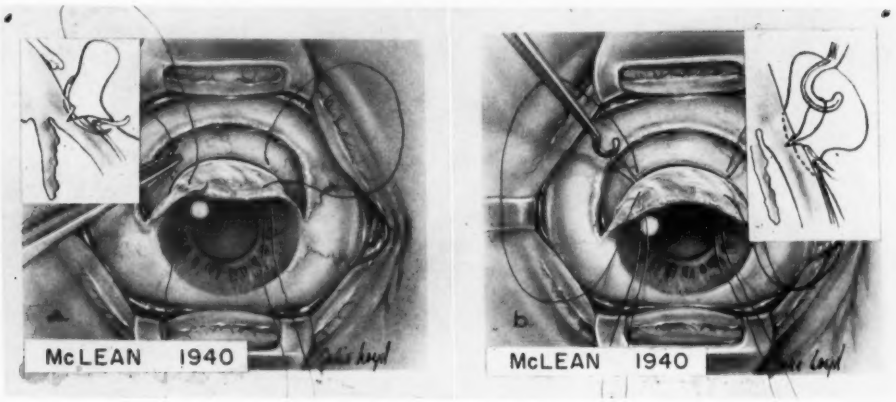


Fig. 6.—McLean corneoscleral closure.

grasped, and with a sawing movement, using the suture much like a Gigli saw, the roof of the tunnel containing the suture was cut to prepare a groove. The upper end of the suture was then inserted at the upper end of the groove to emerge in sclera. A loop was allowed to remain through which the section was made.

6. McLean (1940). McLean,[5] in his excellent article on wound closure, described his technique for preplaced sutures which has become so popular today (Fig. 6). A limbal-based conjunctival flap was prepared, and a groove was made at the limbus with a Lundsgaard knife. One or more sutures could be inserted. McLean usually used two or three. The suture was inserted first through the base of the conjunctival flap. It was then reversed and passed across the groove from the scleral to the corneal side to emerge in clear cornea. The suture was grasped in the gutter, and loops were prepared through which the section was made. McLean's technique admirably fulfills the criteria for an ideal closure. Approximation is accurate and firm. The conjunctival flap is ideal, because it completely covers the corneoscleral incision and the cut edges are sutured well away from the corneoscleral incision.

Evaluation of Corneoscleral Sutures: All corneoscleral sutures insure firmness of closure. All techniques except the Williams suture utilizes preplaced sutures. Weeks partially overcame this objection to the Williams suture by making a partial incision and inserting the sutures in such a way that there could be little lateral overriding. Theoretically, apposition is best with preplaced sutures of the Verhoeff and McLean types, although, practically, excellent apposition can be obtained using the partial section method of Weeks. Of the corneoscleral sutures under discussion, the Kalt suture and the Leech-Sugar modification give the poorest apposition because the arms of the sutures ride over the lips of the wound rather than through the cut edges. The McLean suture has the best conjunctival flap because it completely covers the incision and the cut edges of the conjunctiva are sutured well away from the corneoscleral incision. The flap provided by the Verhoeff technique probably is not ideal because the cut edges of the conjunctiva are at or near the lips of the corneoscleral incision. With the Williams, Weeks, Kalt, and Leech-Sugar closures, the flaps are either absent or very inadequate. Making the section is somewhat difficult with the McLean closure because of the danger of cutting sutures which are placed across a relatively narrow gutter. The sutures, also, are somewhat cumbersome to pull down and tie in a hurry, should an emergency arise, because they may become entangled in the conjunctival flap. All corneoscleral sutures add some inconvenience to cataract extrac-

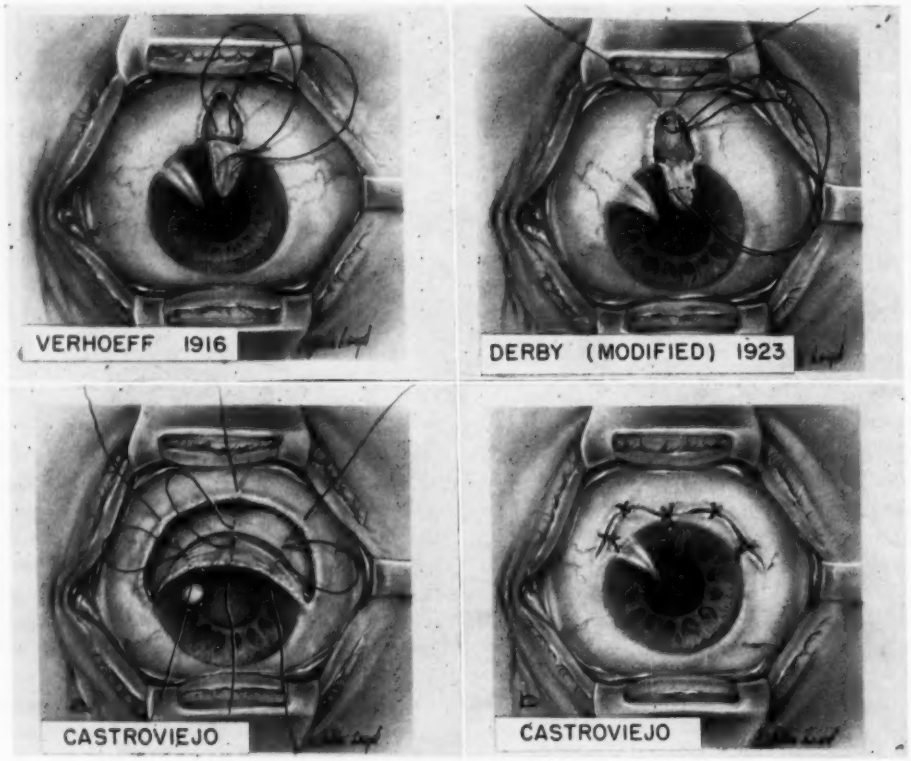


Fig. 7.—Scleral conjunctival closures.

tion, but the advantages far outweigh the disadvantages. Of the group mentioned here, the Verhoeff and McLean wound closures are the most satisfactory.

IV. Scleroconjunctival Sutures.—Three types of scleroconjunctival sutures have been described in the literature.

1. Verhoeff (1916). Verhoeff [19] described the first scleroconjunctival suture (Fig. 7). A small tongue-shaped flap of conjunctiva, about 5 mm. in length, was dissected to the limbus, and a double-armed suture was inserted through superficial sclera exposed by the conjunctival flap, parallel with the limbus about 1 mm. from the limbus. The arms were then passed through the conjunctival flap from its deep surface. Derby,[20] in 1923, modified the Verhoeff suture by passing the double-armed suture first through the conjunctival flap and then through superficial sclera, perpendicular to the limbus, coming out through the cut edges of the bulbar conjunctiva superiorly (Fig. 7). The ends of the suture, placed in this manner, permitted very rapid closure of the wound, which could be accomplished by pulling on the free ends of the suture, since they were directed upward through sclera. Sutures which must be pulled through the corneal flap can cause dangerous gaping of the wound when rapid closure is essential.

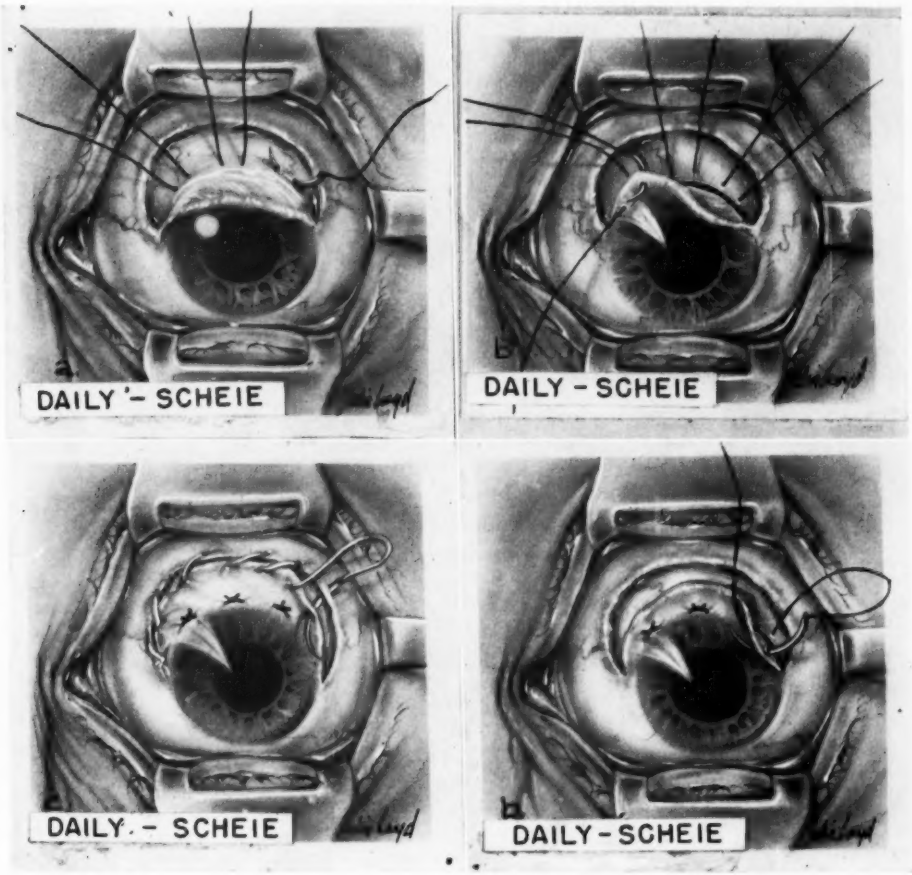
2. Castroviejo. Castroviejo [21] has used a scleroconjunctival suture with a limbus-based flap over the entire length of the incision (Fig. 7). He first turned a 3 mm. conjunctival flap toward the limbus. Three or more sutures were placed through the cut edge of the bulbar conjunctiva and superficial scleral tissue emerging just be-

hind the limbus. They were inserted perpendicular to the limbus. When tied, they closed the conjunctival incision in addition to approximating the edges of the limbal incision. This closure possessed the virtue of simplicity. It offered fairly firm wound closure because the sutures united sclera and limbal tissue at the base of the conjunctival flap. They did not give way like conjunctival sutures placed in more pliable and elastic conjunctiva further away from the limbus.

3. Daily and Daily (1946). Daily and Daily[22] described a very similar suture, and at the same time I, not knowing of their work and believing that I was modifying the closure of Castroviejo, used an almost identical method (Fig. 8). The same type of limbus-based flap was performed, and a bite was taken through superficial sclera very close to and parallel with the limbus. The arms of the suture were then passed through the flap at its attachment to the limbus. These sutures, when tied down, theoretically gave more uniform closure than the Castroviejo type, which were perpendicular to the limbus. The conjunctival flap was closed with a continuous suture. Daily and Daily used one scleroconjunctival suture at 12 o'clock, while I used three.

Evaluation of Scleroconjunctival Sutures: Scleroconjunctival sutures possess the virtue of simplicity. They are easily placed and the section is made with little difficulty

Fig. 8.—Scheie-Daily scleral conjunctival closure.



because adequate space is provided. They can be preplaced and have an excellent conjunctival flap. However, there is no doubt that closure is not as firm or approximation as good as with corneoscleral or corneal sutures inserted through the cut edges of cornea and sclera.

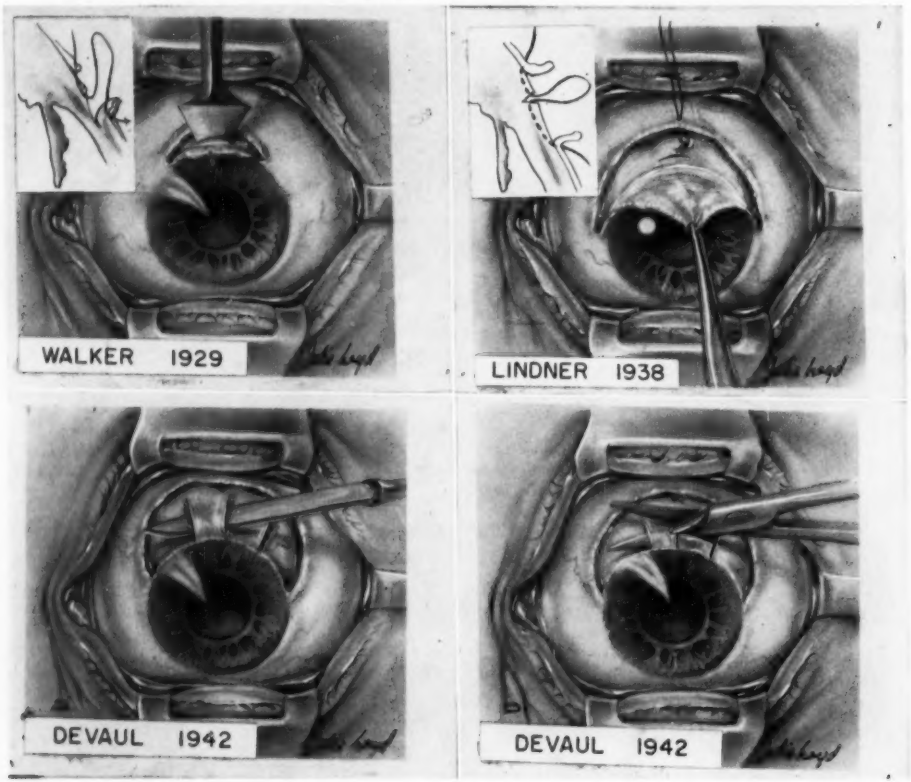
V. Scleral Closures.—Scleral closures all involve the preparation of a scleral flap beneath which a limbal section is made.

1. Walker (1929). Walker[23] dissected a 2 to 3 mm. scleral flap by inserting a keratome or a cataract knife with a guarded tip into the sclera superiorly in such a way as to split the sclera into clear cornea (Fig. 9). A single-arm suture was passed through the lips of the scleral incision as well as through the cut edges of the conjunctiva. He used a knot in the lower end of the suture so that the wound could be closed rapidly by pulling on the upper end of the suture. This idea was contributed by Frisch[24] in 1922.

2. Lindner (1938). Lindner[25] described a scleral suture which utilized the Mendoza principle of making a gutter in the sclera beneath a limbus-based conjunctival flap just behind the limbus at 12 o'clock (Fig. 9). The suture was placed in such a way that, when it was tied, it closed both the scleral and the conjunctival incisions.

3. De Vaul (1942). De Vaul[26] incised the conjunctiva at the limbus to expose the sclera. A cataract knife was held flat on the sclera and inserted into the sclera superficially at approximately 10:30 o'clock to emerge at 12 o'clock to prepare a track. This was repeated from 12 o'clock to 1:30 o'clock. The roofs of the tunnels were

Fig. 9.—Scleral closures.



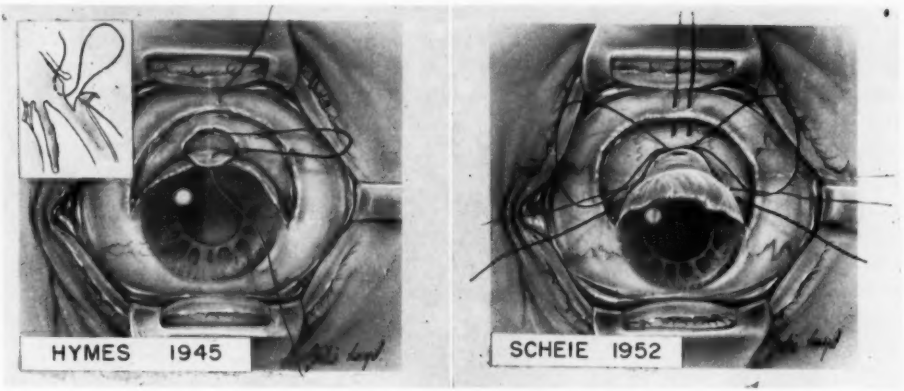


Fig. 10.—Scleral closures.

then divided by a scissors to provide scleral flaps. Sutures were inserted through the edges of the scleral flaps, and both ends passed through the cut edges of the conjunctival flap. When tied, the scleral flaps were closed and the conjunctival flap held in place.

4. Hymes (1945). Hymes,[27] in 1945, described a flap somewhat like that of Walker (Fig. 10). The conjunctiva was divided at the limbus, and the sclera was exposed. A 2 to 3 mm. scleral flap was dissected toward the cornea. A suture was inserted through the cut edges across the scleral incision to include the conjunctiva. When tied, accurate closure resulted.

5. Scheie. At one time, I used a scleral flap dissected in an identical way to that of Hymes. The chief difference was the use of a double-arm mattress suture at 12 o'clock to close the scleral incision (Fig. 10) and a Daily type of scleroconjunctival suture at 10:30 and at 1:30 o'clock for additional support. The free ends of the mattress suture were directed upward, as suggested by Derby. This permitted very rapid closure should necessity arise.

Evaluation of Scleral Flap Closures: All scleral sutures give very firm wound closure. They are preplaced and give excellent apposition. One might, however, question the unwieldiness of a long scleral flap and a possible tendency for the wound to gape, which might be permitted. All of these closures provide an excellent conjunctival flap, and all possess the virtue of simplicity. Section is particularly easy because of the broad space through which to make the incision. Scleral sutures cause less irritation postoperatively than corneoscleral sutures, because they are placed back of the limbus away from sensitive corneal tissue.

Making the Incision

Any discussion of wound closure must include a consideration of techniques of making the incision, because one subject is inseparable from the other. From the time of Daviel until the intracapsular extraction was introduced, the incision customarily was made in the cornea. A larger section was necessitated by the intracapsular method, and, as a result, the incision had to be moved to the limbus and had to be a half-section. With the incision located at the limbus, the use of limbus-based conjunctival and scleral flaps became possible.

The usual incision is somewhat beveled. Atkinson[28] has suggested making a corneal incision with an exaggerated bevel to obtain firmer approximation (Fig. 11). The degree of bevel greatly affects the size of the incision. When the bevel is increased, the inner corneal lip encroaches to a greater degree upon the wound aperture and may cause difficulty in delivery of the lens, because of the variation in the size of the opening and the overhanging ledge which

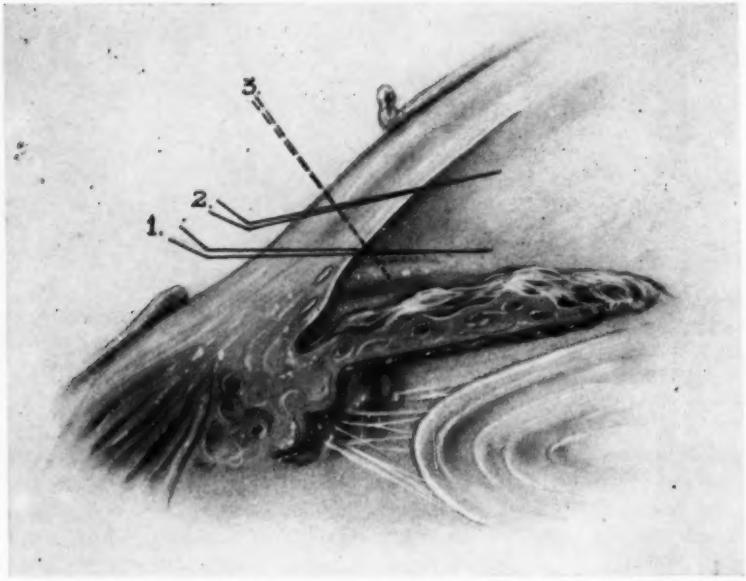


Fig. 11.—Corneal ledge with (*1*) normally beveled limbal incision, (*2*) more highly beveled corneal incision (after Atkinson), and (*3*) perpendicular corneal incision (after Atkinson).

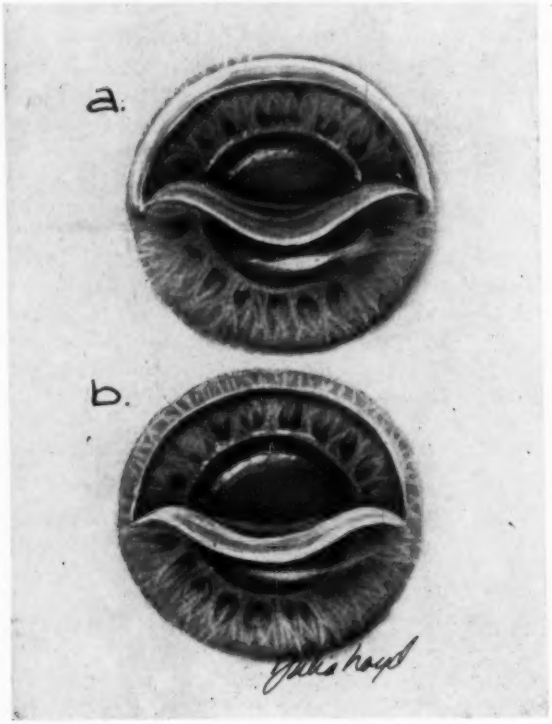
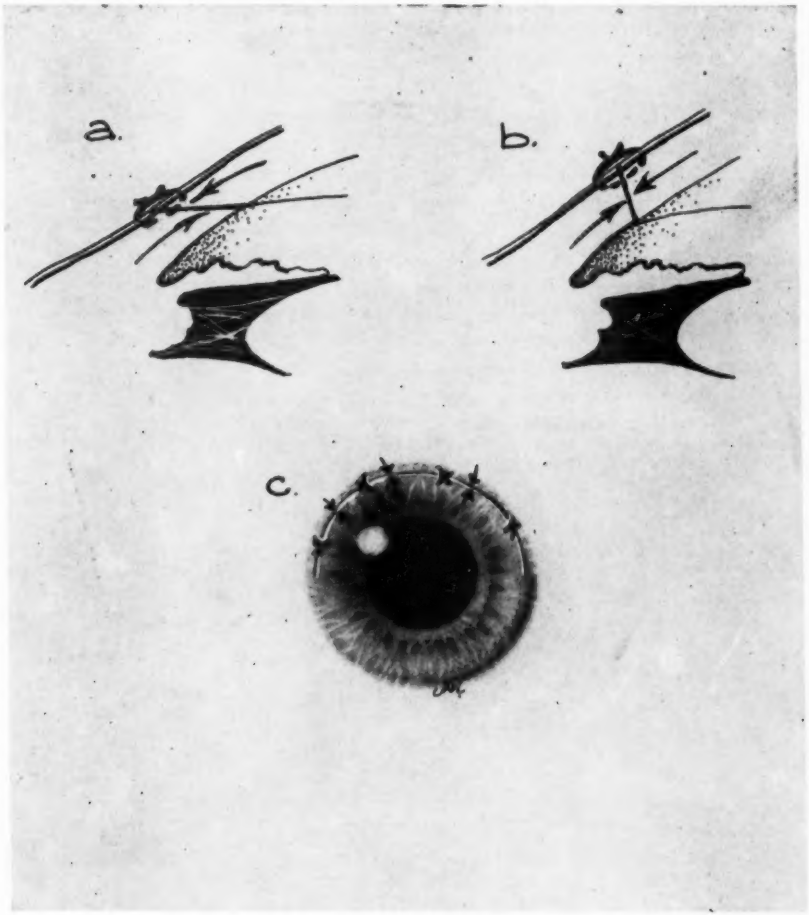


Fig. 12.—Corneal ledges of identical size (*a*) with normally beveled limbal incision and (*b*) with perpendicular incision well within clear cornea.

results (Figs. 11 and 12). If the bevel is diminished and the incision approaches the perpendicular, the ledge is smaller and the aperture is larger.

When the incision is completely perpendicular, cataract extraction is possible, even with incision placed well within clear cornea. The inner lip protrudes no more than with a moderately beveled limbal incision, and, as a result, the size of the aperture is not affected. I first used this type of incision for cataract extraction after filtering operations for glaucoma,[20] to avoid disturbing the filtering cicatrix. Experience showed that this technique was accompanied by very few wound complications and that it was safe to execute. Experience with keratoplasty, where wound edges are also perpendicular, had shown that reformation of the anterior chamber and choroidal detachment is extremely rare. Encouraged by these observations, a technique with a perpendicular incision was developed for routine cataract extraction. This incision now has been used in over 1,000 uncomplicated cataract extractions during the last five years in my private practice and in the clinic at the Hospital of the University of Pennsylvania.

Fig. 13.—Mechanics of perpendicular wound closure; (*a*) and (*b*), less tendency to override with perpendicular incision; (*c*), arrows indicate tendency of flat surfaces of incision to seal with postoperative edema of wound.



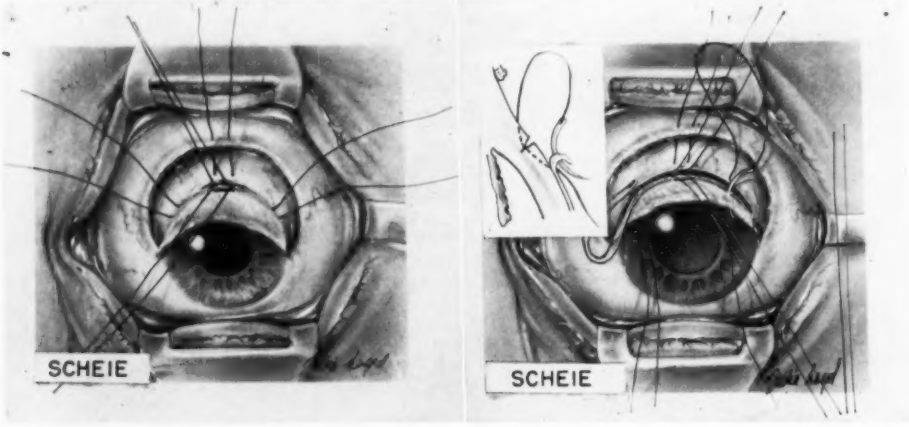
Perpendicular incisions are mentioned rarely in the literature. I have seen Castroviejo[21] make perpendicular incisions for routine cataract extractions. McCulloch[30] has told me that he uses an incision which is partially perpendicular for extraction, and Barraquer[31] has devised a special instrument for making perpendicular incisions. Katzin and Peyton,[32] in their book on ophthalmic surgery, mention the use of a perpendicular incision. Theoretically, such an incision should prevent overriding of wound edges, because flat surfaces held in contact by sutures should press even more firmly together with onset of postoperative edema of the wound edges. This would probably also be true even between the sutures, if an adequate number are inserted (Fig. 13). Hyphemia should also be rare, because the incision is entirely within avascular corneal tissue.

Numerous articles have appeared in the literature on the choice of instruments for making the incision. The standby of older ophthalmologists was the Graefe knife, which had supplemented knives of the Beer type. In recent years, use of the keratome and scissors has become a thoroughly accepted technique. Arguments can and have been raised in favor of one method or the other, but it is generally concluded that excellent incisions can be made by either method.

My Present Technique of Incision and Wound Closure

My present technique represents a gradual transition from one type of wound closure to another, adopting with each change any desirable features of previously used techniques. Scleroconjunctival closures were used and discarded. The next step was a scleral flap, retaining two scleroconjunctival sutures, one nasally and one temporally, as supporting sutures. A mattress suture was inserted in the scleral flap above. Although iris prolapse was extremely unusual, a rather large number of choroidal detachments accompanied the use of the scleral flap technique. It was thought that the long scleral flap might lead to bulging of the wound, and so the incision was moved up to the limbus, the scleral closure then becoming corneoscleral (Fig. 14). The suturing technique was identical to that of the scleral flap. Later two postplaced McLean sutures were substituted for the scleroconjunctival sutures and supported at each side, and eventually the technique about to be described was adopted. It includes a corneal incision, one McLean suture at each side, and a mattress suture superiorly, these

Fig. 14.—Scheie corneoscleral closure (*a*) with mattress suture at 12 o'clock and scleroconjunctival sutures at 10:30 and 1:30 o'clock; (*b*) two postplaced McLean type sutures substituted for the supporting scleroconjunctival sutures.



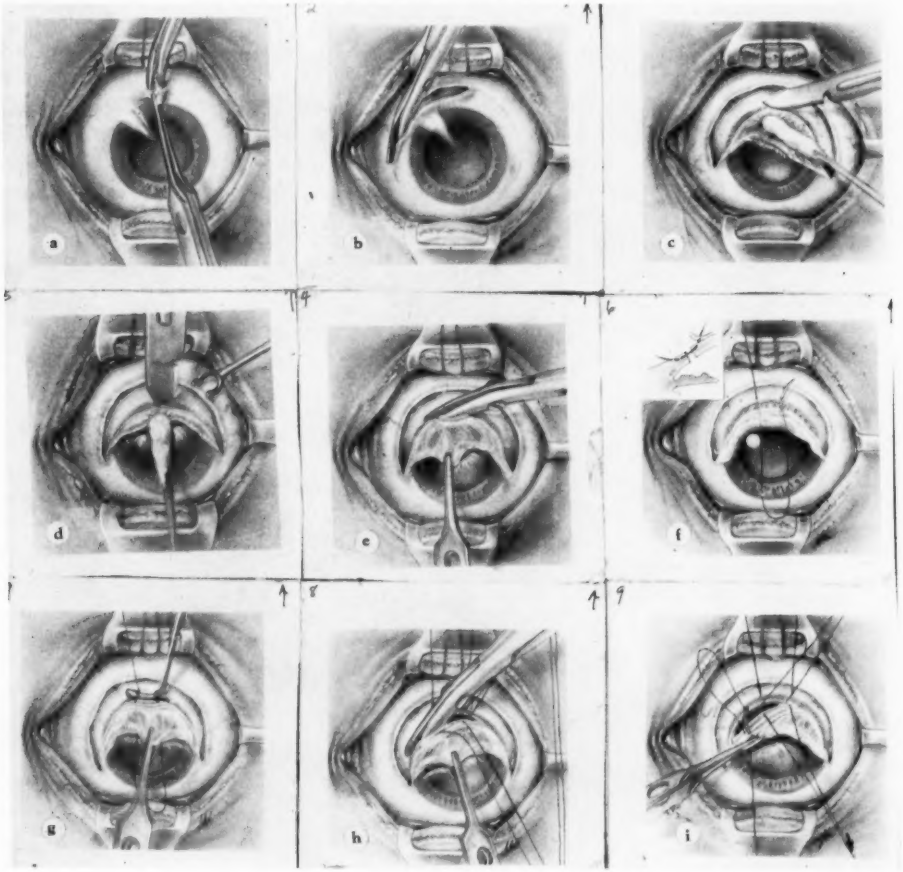
latter converted by splitting each arm to two McLean sutures.

Although the sutures are postplaced, experience with this technique indicates that adequate accuracy is obtained. The perpendicular incision prevents overriding of the cornea, a contention which is supported by the rare wrinkling of the corneal flap upon tying the sutures and by a rather low astigmatic error. I have used this technique for more than five years with no iris prolapses. Delayed choroidal detachment with shallow chambers is extremely rare as compared with previous experience with a scleral flap. No hyphemia of consequence has occurred.

Procedure.—A 3 mm. conjunctival flap is reflected to the limbus (Figs. 15*a* and *b*). The flap is then dissected into the cornea, which is split for 1 to 2 mm. (Fig. 15*c*).

A No. 15 Bard-Parker blade, on a small handle, is used for splitting. The eyeball is prevented from rolling, while the cornea is being split, by fixation obtained with a firmly tied superior rectus suture and pressure upon the globe with a moistened cotton applicator placed on the reflected conjunctival flap. The applicator simultaneously aids in fixing the globe and retracts the conjunctival flap to aid in corneal splitting. A line of cleavage for splitting is most easily found nasally and temporally rather than above. Dissection, therefore, is started at one of these two sites and carried around

Fig. 15.—My current technique of wound closure.



the upper half of the globe. Hemostasis is obtained by a Hildreth cautery.

The anterior chamber is entered by an *ab externo* type of incision using the tip of a Bard-Parker knife (Fig. 15*d*). An assistant retracts the split cornea and the conjunctival flap at 12 o'clock, and the eye is fixed with a Burch scleral pick held by the operator. A perpendicular incision is made 0.5 to 1 mm. within clear cornea at 12 o'clock, near the insertion of the split portion of the cornea. The opening is enlarged a few millimeters nasally and temporally with a scissors (Fig. 15*e*). A double-arm suture is inserted, after the principle of Weeks, near each extremity of the cut to insure accuracy of placement (Fig. 15*f*). A loupe is used for magnification to aid in meticulous placing of sutures. The arms of the mattress suture are inserted 4 to 5 mm. apart. The needle enters the cornea at the base of the conjunctival corneal flap and emerges through the superficial layers of the cut edge of the corneal flap, never deeper than one-third to one-quarter the thickness of the cornea. Next it is passed through the scleral edge of the incision at the same depth and emerges from the sclera about 2 mm. beyond the limbus. The other arm is inserted likewise, and the arms of both sutures are pulled from the incision by a muscle or scleral hook and withdrawn to form two loops (Fig. 15*g*). A hemostat is placed through the loops and clamped to the drapes near the center of the eyebrow in such a way that the tip of the hemostat rests over the arms of the loops and the free ends of the suture (Fig. 16). The weight of the hemostat is sufficient to serve as countertraction when an assistant pulls on the lower arms of the loops to retract the iris during the iridectomy.

After the double-armed suture is placed, the corneal wound is enlarged, with care taken to hold the scissors at right angles to the cornea to produce a perpendicular incision (Fig. 15*h*). A specially designed scissors is used which has a heavy shank to insure clean cutting of tissues without crushing and buckling and a blunt tip to prevent buttonholing of the iris.[33] The scissors are made in right- and left-handed instruments in such a way that the inner blade is forward toward the center of the

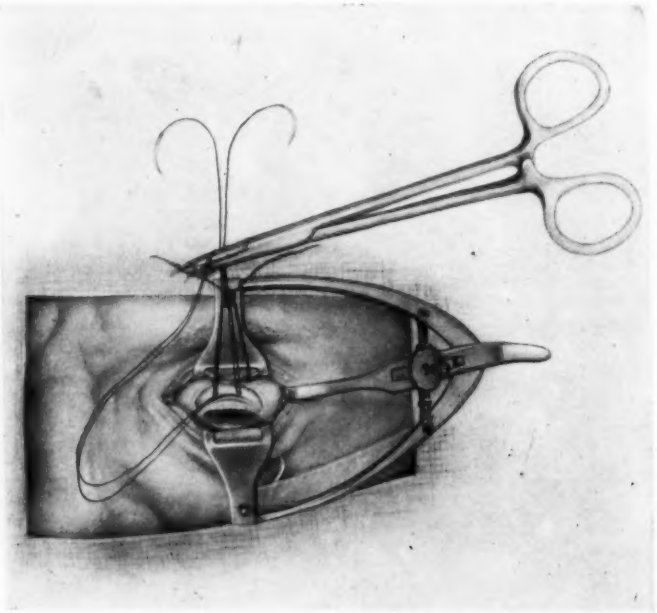
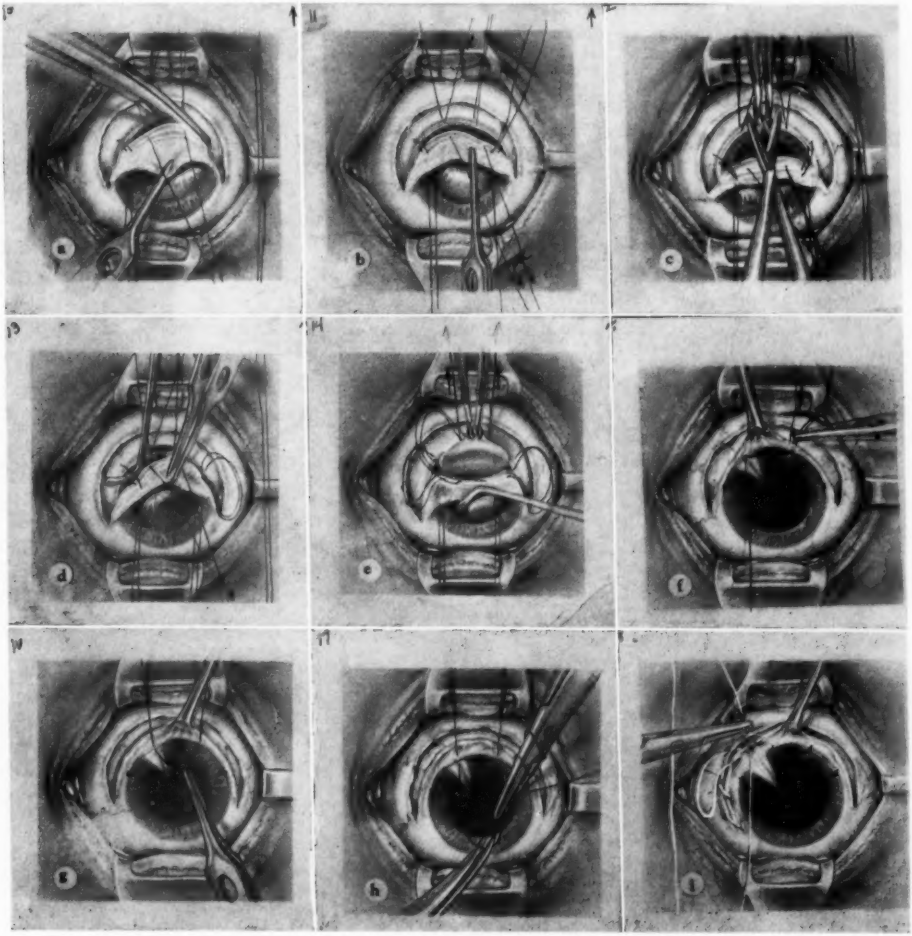


Fig. 16.—Hemostat through loops and resting upon free ends of suture.

cornea. This places the incision about 0.5 mm. further toward the corneal side than would be true if the blades were reversed.

As the incision is enlarged, a postplaced McLean type of suture is inserted across the corneal incision at 10:30 and 1:30 o'clock (Figs. 15*i* and 17*a* and *b*). The needle enters the cornea near the base of the conjunctival corneal flap, at a point adjacent to the attachment of the split-off cornea to the cornea proper. Sufficient fixation for inserting this suture is obtained, when a sharp Grieshaber type of needle is used, by grasping the conjunctival flap with a forceps and holding it up under some tension. Raising the flap in this manner provides a groove at its base which helps to guide the needle properly into the cornea. After the needle emerges through the superficial portion of the perpendicular cut edge of the corneal incision, the tip is pushed through for 4 or 5 mm. Alignment for the second bite into the scleral side of the corneal incision is obtained by gentle back-pressure on the surface of the cornea and sclera adjacent to the incision, which causes a slight indentation that can be seen with a loupe. The needle is then pulled back and

Fig. 17.—My current technique of wound closure (continued).



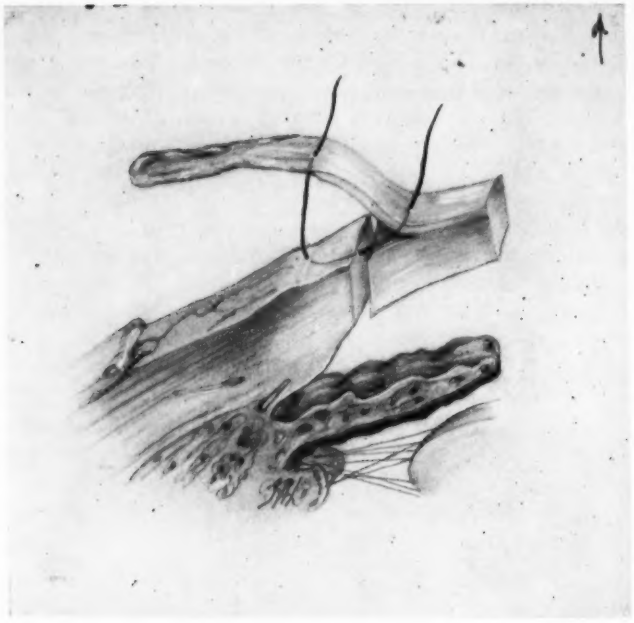
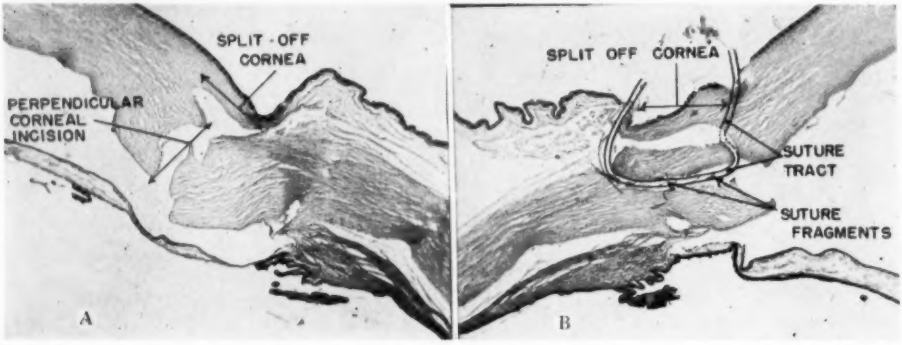


Fig. 18.—Perpendicular incision covered by corneal flap, McLean type of suture in position.

passed through the cut edge on the scleral side of the incision. The needle must emerge about 1 mm. back of the limbus, so that, when tied, the layer of split-off cornea, which is encircled by the suture, is not wrinkled. The needle is reversed and brought back through the base of the conjunctival flap at its junction with the split-off cornea. When the suture is tied, not only are the cut corneal surfaces closed but also the layer of split cornea is pressed firmly into position over the incision (Figs. 18 and 19). A corneal closure is thus obtained which resembles a carpenter's "half lap" joint.

Exposure for iridectomy is obtained by an assistant, who retracts the corneal flap by inserting a muscle hook into the lower portion of the loops of the mattress suture and retracting them downward (Fig. 17*c*). After iridectomy, sufficiently large loops are formed in the nasal and temporal sutures

Fig. 19.—(*a*) Cross section of donor eye in which perpendicular corneal incision has been made beneath a corneal flap. (*b*) Section through suture identified by fragments and superimposed sketch.



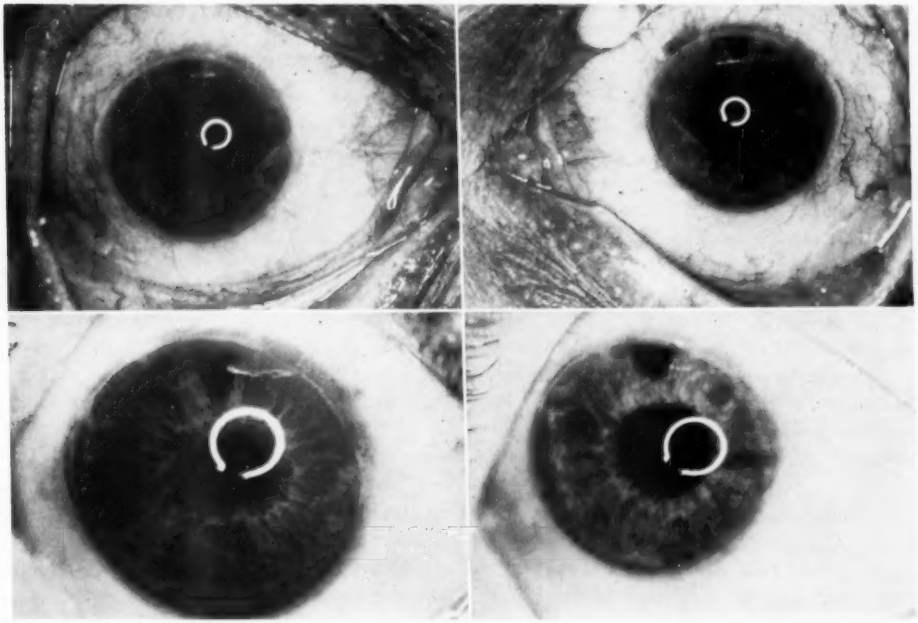
to permit delivery of the lens (Fig. 17*d*). These loops are placed on the sclera. The ends of the mattress suture at 12 o'clock are pulled up to leave loops only long enough to permit delivery of the lens (Fig. 17*e*). After delivery, the mattress suture is pulled up immediately to close the wound. The arms of the suture are then held taut by an assistant while the two McLean supporting sutures are tied. Once these are tied and the wound is partially closed, the needles of the mattress suture are reversed and passed from the deep side of the conjunctiva at the attachment of the conjunctival flap to the cornea (Fig. 17*f*). The loop at the end of the mattress suture is grasped with a forceps and pulled downward until about 1 or 2 in. protrudes (Fig. 17*g*). The loop is divided, creating two separate sutures of the McLean type (Fig. 17*h*). These are tied, and the conjunctival incision is closed with a 000000 mild chromic surgical absorbable (gut) suture (Fig. 17*i*). Only the eye operated on is covered, and the patient is allowed to become ambulatory whenever the effects of sedation have subsided.

Comment

The history of incision and wound closure in cataract extraction has been briefly reviewed. Some of the more basic types of closure have been discussed, and outstanding features are pointed out. Individual closures are evaluated by criteria established by McLean. He stated that an ideal wound closure should (1) provide firm approximation; (2) be preplaced; (3) furnish good apposition, with sutures passing through the cut edges; (4) provide a good conjunctival flap to cover the incision, and (5) not be too complicated.

Firmness of closure and good apposition are the most important of these criteria, with a conjunctival flap next in order. Preplacement of sutures is of less importance today than in the past because of good anesthesia and akinesia, although preplacing sutures insures accuracy of apposition. Simplicity is of secondary concern, because

Fig. 20.—Postoperative photographs showing scar of corneal incision within limbus.



any closure can be used with little or no difficulty with the sharp needles available today.

In searching for an ideal wound closure, we find five general types from which to choose: (1) conjunctival, (2) corneal, (3) corneoscleral, (4) scleroconjunctival, and (5) scleral. Conjunctival wound closures need not be considered seriously, because they fail to give firm closure and they provide poor apposition. Corneal incisions are rarely used today with the intracapsular extraction and are, therefore, important chiefly because of their historical interest. We find in use today scleroconjunctival, scleral, and corneoscleral closures (Table).

Selection of Most Suitable Suture

	McLean Criteria *				
	Firmness	Preplaced	Apposition	Conjunct. Flap	Simplicity
Daily-Schele-Castroviejo sclerocon-junctival	++	++++	+	++++	++++
Williams	++++	0	++	0	++++
Weeks	++++	+++	+++	0	+++
Kalt (Leech-Sugar)	++++	++++	+	+	++++
McLean	++++	++++	++++	++++	++
Verhoeff tunnel	++++	++++	++++	+++	++
Scleral	++++	++++	++++	++++	++

* Rated in effectiveness from 0 to ++++.

Scleroconjunctival closures fail to provide firm or accurate wound closure. They do provide an excellent conjunctival flap; they can be preplaced, and they are simple to use. Scleral closures are more ideal in that they provide firm closure with sutures placed across the lips of the wound, but I have found that they are accompanied by a high incidence of choroidal detachment, probably due to fistulization along the lips of the unwieldy scleral flap. This long flap probably permitted some gaping of the wound.

Corneoscleral closures more nearly approach the ideal. The Williams type of suture gives firm closure, but it is postplaced and has no conjunctival flap. The Weeks technique improves its apposition greatly by permitting placement of the sutures as the wound is enlarged. The Kalt suture provides firm closure, but, because it is a bridge-type suture, it overrides the wound and fails to provide good apposition. Sutures of the McLean or the Verhoeff tunnel type most nearly approach the ideal. Each gives firm and accurate closure. Each is preplaced. The conjunctival flap is probably better with McLean sutures. Each is somewhat complicated, and some difficulty is encountered in their use, but certainly their value overcomes this objection.

My current technique of incision and wound closure was described. It embodies features taken from several previously described closures. A somewhat unorthodox incision is made by first preparing a conjunctival limbus-based flap, which is then split into the cornea for 1 to 2 mm. A perpendicular incision is made into the anterior chamber through clear cornea beneath the portion of split-off cornea near its attachment. Because the incision is perpendicular, the overhanging ledge of corneal tissue is no greater than with a limbal incision of ordinary bevel. Postplaced sutures of the McLean type are used. When the sutures are tied, the flat surfaces of the incised lips are approximated, and because the edges are flat overriding is prevented. At the same time, the incision into the anterior chamber is covered by a flap of split-off cornea. Sealing of the wound is usually prompt. Only the eye operated on is covered. The patients may become ambulatory as soon as the preoperative sedation wears off. Delayed re-formation of the anterior chamber and choroidal detachment are extremely rare. In five years of experience with over 1,000 patients, no hyphemia of sufficient degree to interfere with the outcome of the operation has occurred. No iris prolapse has been encountered. The astigmatic error is 1.15 D., averaged in 100 consecutive patients, as compared with 2.04 D., with a similar series, using my scleral flap technique.

Summary

Basic types of incision and wound closure for cataract extraction are compared. My present technique of using a perpendicular corneal incision, made beneath a small corneal flap, is discussed.

313 S. 17th. St. (3).

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Sympathectomy and Immunologically Induced Bilateral Eye Reactions in the Rabbit

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Introduction

If a sterile foreign protein is injected into rabbit vitreous and about two weeks later the same antigen is given intravenously, an acute aseptic inflammatory reaction not only occurs in the uveal tract of the eye into which the material was previously injected but may also occur to less degree in the contralateral eye. These bilateral eye reactions have been experimentally produced and described by Woods,[1] Guillery,[2] von Szily,[3] Marchesani,[4] Seegal and Seegal,[5] Schlaegel,[6,7] Foss,[8] Schlaegel and Wilson,[9] and Schmedtje.[10,11]

Different explanations have been proposed for bilateral eye reactions following the aggravation of only one eye. In the case of the experimentally produced reactions just described in the rabbit, an explanation emphasizing local tissue antibodies was offered by Foss.[8] He suggested that the original antigen, injected intraocularly, not only sensitizes the cells of the eye into which the material is injected but also passes via the blood stream to the eye not given an injection, "electively sensitizing" it. Thus the cells of both eyes contain antibodies and may react to any subsequent systemic introduction of antigen. While admitting the possibility of local tissue antibodies, Schlaegel and Wilson[9] interpreted their results in terms of circulating antibodies combining with persisting antigen at the site of the elective sensitization.

Elschnig[12] and Woods[13] originally proposed the explanation implicating uveal pigment release as the cause of similar bilateral eye reaction. Their evidence indicated that traumatization of the eye by any one of a number of different factors can release uveal pigment into the circulation; this organ-specific pigment stimulates the formation of systemic antibodies, which are capable of reacting with the uveal pigmented cells in the contralateral eye, causing a "sympathizing" reaction.

The explanation of bilateral eye reactions in rabbits as due to a basic mechanism involving infectious processes was first proposed by Guillery.[14] Different suspect organisms have been worked on by a number of investigators, whose work is reviewed by Alvaro.[15] From this viewpoint, specific viruses or bacteria readily attack an eye traumatized, for example, by an anaphylactic tissue reaction. The organisms adapt to the traumatized eye tissues and subsequently find the contralateral eye preferentially suitable for further parasitization, resulting in a bilateral reaction.

Other investigators have interpreted experimentally produced bilateral eye reactions as due solely to neural reflexes. Denig[16] cites his experiments with rabbits and dogs and reviews the works of several other supporters of this explanation. He theorizes that inflammatory processes in one eye, of whatever cause, initiate sensory impulses that cause reflex "sympathetic irritation" in the other eye.

A review of these numerous and conflicting explanations brings forth the realization

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This investigation was begun in the Department of Anatomy, St. Louis University School of Medicine, and completed in the Department of Anatomy, Harvard Medical School.

Early phases of this investigation were presented at the Southern Section of the Association for Research in Ophthalmology, St. Louis, Nov. 10, 1954, and at the American Association of Anatomists, Philadelphia, April 8, 1955.

that the basic mechanism is still not clearly understood for the experimentally produced bilateral eye reaction in the rabbit. This applies particularly to those reactions that result from severe antigen-antibody reaction in one eye.

This investigation was designed to find what role neural reflexes may have in bilateral rabbit eye reactions following severe antigen-antibody reactions in one eye. The finding is that intact sympathetic innervation is necessary for the contralateral reaction to occur. This is interpreted to indicate either that sympathectomy of the contralateral eye radically modifies any antigen-antibody reaction in the eye or, more likely, that sympathectomy interrupts the motor part of a reflex arc solely responsible for the contralateral reaction.

Methods

Establishment of Gross and Histological Basis for Comparing Effects of Sympathectomy.—The right eyes of 10 control-group rabbits were prepared with several drops of 1% procaine. The ensuing local anesthesia permitted immobilization of the eye by means of grasping the sclera with fine-toothed forceps. A 26 gauge needle on a tuberculin syringe was introduced through the sclera just posterior to the limbus into the vitreous, and 0.2 ml. of a 15% solution of human albumin (product of Cutter Laboratories) was injected. Fourteen days later 5.0 ml. of the same solution of albumin was injected into the marginal vein of the ear. Both sensitized and contralateral eyes were inspected daily for superficial gross signs of uveal inflammation. On the sixth day following intravenous challenge, the rabbits were killed by air embolism. All eyes were fixed in Zenker's fluid, and scattered serial sections were stained with hematoxylin and eosin and examined with the light microscope.

Observation of Effect of Sympathetic Denervation upon Reaction of Eye Given Injection to Intravenous Challenge.—The right eyes of four rabbits were sympathectomized by removal of the right superior cervical sympathetic ganglia. Intravenous pentobarbital (Nembutal) (26 mg. per kilogram of body weight) was used as the anesthetic. Care was taken to free the adjacent carotid arteries of all postganglionic fibers ascending from the middle and inferior cervical ganglia. After complete recovery these four rabbits were subjected to the same procedures as the control group, namely, intravitreous injection of human albumin into the right eye and intravenous challenge 14 days later, followed by gross and histological examination of both eyes.

Observation of Effect of Sympathectomy upon Reaction in Uninjected, Left Eye.—The left eyes of eight rabbits were sympathectomized by removal of the left superior cervical ganglia. After complete recovery these eight rabbits were subjected to the same procedures as the above groups of rabbits, namely, right eye intravitreous injection, intravenous challenge, and gross and histological examination of both eyes.

Results

Intact Innervation to Both Eyes.—Right eye intravitreous sensitization, followed 14 days later by intravenous challenge, caused a grossly visible inflammatory reaction in the right eye in every control-group rabbit. This reaction began to appear within two hours after the intravenous challenge of antigen and reached a maximum intensity within three days. These right eyes showed a pericorneal engorgement of blood vessels. The radial blood vessels in the iris were

Fig. 1.—Superior view of normal rabbit eyes. Lower part of picture is anterior. Construction of large amounts of orbital smooth muscle under sympathetic stimulation causes an exophthalmos and squeezes shut some venous blood vessels.

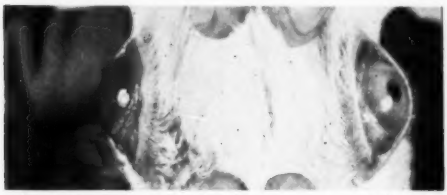

Fig. 2.—Same view as Figure 1; a rabbit with all nerves to both eyes intact, showing a severe antigen-antibody reaction in the right eye and a grossly visible mild inflammatory reaction in the left eye.

visibly engorged, and small hemorrhagic areas became visible (Figs. 1, 2). A cloudy precipitate could be seen in the aqueous humor, and the conjunctival membranes became red and appeared inflamed. The effects of the antigen-antibody reaction were visible in the freshly fixed and dissected eye (Fig. 4). Histological examination of these eyes revealed engorgement of most of the small blood vessels in the eye and distinct hemorrhagic areas in the iris and ciliary body. There was a massive infiltration of the whole uveal tract with mononuclear cells consisting of monocytes, lymphocytes and plasma cells (Fig. 5). Polymorphonuclear cells were few in number or completely absent. The cellular exudate swelled the whole uveal tract to an abnormal thickness. This exudate appeared to have broken out through the epithelial lining of the ciliary processes. The retina appeared disorganized and in places was detached from the swollen choroid. Exudate and mononuclear cells were present (Fig. 6). Perivascular infiltration was also present in the sclera.

In 8 of the 10 control-group rabbits there was histological evidence of a mild inflammatory reaction in the contralateral eye. One of these positive contralateral reactions was severe enough to be observed by gross inspection. The radial and circumcorneal blood vessels were visibly engorged (Fig. 2). All eight of these positive contralateral reactions were characterized histologically by an abnormal increase in the number of lymphocytes, monocytes, and plasma cells in the uveal tract (Fig. 7). There was a mild thickening of the choroid due to the round-cell infiltration and exudation. Many small blood vessels showed mild perivascular infiltration. This reaction, while evident, was in no way as severe as the infiltration, exudation, hemorrhage, and distortion seen in the reaction of the sensitized eye. In two of the control-group rabbits there was no histological evidence of a contralateral reaction.

Fig. 3.—Same view as Figures 1 and 2; a rabbit with the left eye sympathectomized, showing a severe antigen-antibody reaction in the right eye and no reaction in left eye.

Fig. 4.—Fixed and dissected eye, showing gross effects of antigen-antibody reaction in iris of an eye sensitized by intravitreous injection.

Sympathectomized Sensitized Eye.—In the second group of rabbits, that in which the right eyes were desympathectomized before the albumin was injected intravitreously, the intravenous challenge caused a very severe inflammatory response in all of the sensitized right eyes. Histological inspection of the uveal tracts of all contralateral eyes revealed a mild inflammatory reaction characterized by an abnormal round-cell infiltration, exudation, and hyperemia.

Sympathectomized Contralateral Eye.—In the third group of rabbits, that in which the left eyes had been previously sympa-

Fig. 5.—Section at angle of iris of sensitized eye six days after intravenous challenge. Note massive round-cell infiltration of iris. Exudate both anterior to iris and around ciliary processes also contains mononuclear cells.

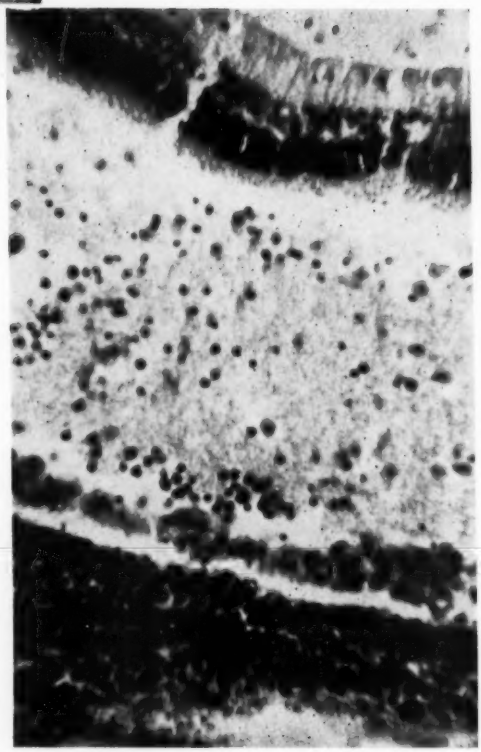

Fig. 6.—Section of retina, showing exudation and round-cell infiltration, especially of plasma cells, resulting from an antigen-antibody reaction in a sensitized eye.

Fig. 7.—Section of base of iris and ciliary body in a sympathectomized unsensitized contralateral eye, showing an abnormal hemostasis and round-cell infiltration.

thectomized, intraocular sensitization of the right eye followed by intravenous challenge again caused a severe inflammatory reaction in the right eye. However, in not one of the eight contralateral left eyes was there any gross (Fig. 3) or histological evidence of a mild inflammatory reaction.

Comment

Intact Innervation to Both Eyes.—The results in the control group of rabbits are similar to the findings of several previous investigations.[7-9] The 80% of the control-group rabbits that have a contralateral reaction is comparable to the percentage found by Schlaegel [7] with use of the same 2-week time interval between sensitization and challenge by means of a shocking dose of antigen. By repeated intravenous challenge with antigen, he was able to elicit the bilateral reaction in 100% of his rabbits. While the same procedure would probably have yielded a 100% bilateral reaction in the control group, it was believed that the single large shocking dose of human albumin gave results of sufficient intensity to establish the validity of the reaction and a sufficiently large number of positive contralateral reactions to provide a valid basis for comparing the effects of sympathectomy.

Sympathectomized Eye Given Injection. The results in the second group of rabbits showed that sympathetic denervation had little effect on the antigen-antibody reaction in the eye that was sensitized by intraocular injection. Presumably there were antibodies in or on the cells of the sensitized eye that had a very strong affinity for the antigen that later reappeared through the blood vessels entering the eye. Earlier sympathectomy of these eyes had deprived the blood vessels of their vasoconstrictor fibers. Yet lack of vasoconstrictor fibers did not modify the local reaction in the sensitized eye. This could be because the blood vessels had regained their own ability to constrict and relax during the time lapse after sympathectomy (Kuntz [17]). However, the reactions in these sensitized eyes were so intense that any small effects could have been masked.

It is believed significant that a contralateral reaction still occurred in most of these second-group rabbits. Sympathectomy had not interrupted the sensory fibers from the eye to be sensitized because the sensory fibers do not pass through the cervical sympathetic trunk. In addition, all the autonomic fibers to the contralateral eye were still intact. Both the afferent arm from

the sensitized eye and the efferent arm of a possible reflex arc to the contralateral eye were still intact.

Sympathectomized Contralateral Eye.—In regard to the third group of rabbits, the results indicate that sympathectomy of the contralateral eye radically modifies subsequent contralateral reactions. Two general interpretations may be given to the finding that the mild contralateral reaction fails to occur in a sympathectomized eye. One is that lack of sympathetic fibers to the contralateral eye prevents or depresses any antigen-antibody reaction in that eye. The other is that sympathectomy of the contralateral eye interrupts the efferent part of a reflex arc responsible for the reaction.

Effects of Denervation on Antigen-Antibody Reaction: On the basis of the following evidence, relatively weak support is to be found for the explanation that sympathectomy depresses an antigen-antibody reaction in the contralateral eye:

First, there is no evidence offered here or in the investigations of similar bilateral reactions that the mild contralateral reaction is really due to an antigen-antibody reaction.[7-9] The often-used term "elective sensitization" of the contralateral eye implies that more antigen has reached this eye than elsewhere or that for some unknown reason more cellular antibodies have been formed here than elsewhere. Evidence that either really occurs is lacking. Foss [8] claimed to be able to detect serologically the antigen in the aqueous humor of the contralateral eye. However, the blood-aqueous barriers and the fast rate of aqueous circulation would not favor an accumulation of antigen in the aqueous of the contralateral eye (Langham [18]). Nor would any other known factors favor the contralateral eye cells receiving a longer exposure and greater sensitization to the circulating antigen than any other cells of the body.

Mention should be made of the optic nerve route for antigen transport that has been suggested as the anatomical basis for elective sensitization. Alvaro [15] cites the work of Deutchman and others, who concluded from their experiments that bacteria and viruses can move through the lymph channels and connective tissue surrounding the optic nerves. A pathway would thus exist along the optic nerve of one eye, across the optic chiasma, and back along the optic nerve of the other eye. But while the possibility of this optic chiasma route has been demonstrated for propagating bacteria and viruses, evidence is still lacking that it could be a means for the diffusion of antigen.

Second, sympathectomy in the second group of rabbits did not visibly depress the known antigen-antibody reaction in the sensitized eye. It was mentioned above that any effect could have been masked by the severity of the reaction, but one must consider the weight of evidence in the literature, reviewed by Urbach and Gottlieb,[19] that indicates that sympathectomy does not depress local antigen-antibody reactions.

Third, it would be difficult to support an explanation that sympathetic denervation depresses an antigen-antibody reaction in the contralateral eye because of altered vasomotor innervation. A large amount of experimentatl data, reviewed by Kuntz,[17] indicates that following cervical sympathectomy a brief hyperemia and increased intraocular pressure occur within the eye. Later both these effects disappear because the blood vessels regain their inherent tonus. Thus, any antigen-antibody reaction in the sympathectomized contralateral eye would be occurring in tissue the vascular milieu of which is presumably the same as in a normally innervated eye.

As a fourth point, sympathectomy would, if anything, augment an antigen-antibody reaction, according to a large amount of evidence reviewed by Kuntz.[17,20] In considering the effects of the autonomic nervous system on the immune tissue reaction he concludes that the most characteristic manifestations of these reactions appear to be causally related to a heightened parasympathetic or cholinergic activity. According to this, if the contralateral reaction in the "electively sensitized" eye was due to an

antigen-antibody reaction, we would expect that sympathetic denervation would augment and intensify such a reaction. The experimental results indicate clearly that it does not. This weakens any explanation that attributes the contralateral reaction to an antigen-antibody reaction.

Reflex Mechanism as Explanation of Contralateral Reaction: In contrast to the preceding, relatively strong support is available for the explanation that sympathectomy of the contralateral eye interrupts the efferent part of a reflex arc and thereby prevents the occurrence of the contralateral reaction.

In the first and second groups of rabbits, the nerve pathways for a possible reflex reaction are intact and the contralateral reaction does occur. In the third group of rabbits, these pathways are interrupted and the reaction does not occur. The sensory fibers from the sensitized eye originate in nerve plexi in the iris, choroid, and cornea. They pass from the eyeball via the long and short ciliary nerves to the nasociliary branch of the ophthalmic division of the fifth cranial nerve. In addition, there are the special sensory fibers of the optic tract, originating in the retina, that could be receptive to pressure changes. While visceral sensory fibers from thoracic and abdominal viscera pass to the central nervous system via the sympathetics, this is not the case in the eye where all the visceral sensory nerves pass to the central nervous system via the fifth cranial nerve (Kuntz [17]). Therefore, cervical sympathectomy in the second group of rabbits did not interrupt the sensory fibers from those eyes and complete reflex pathways were intact from the sensitized eye to the contralateral eye. The motor part of a possible reflex arc from the sensitized to the contralateral eye would consist of sympathetic fibers. The sympathetic fibers originate from the second thoracic level of the spinal cord and pass via the sympathetic trunk and ganglia, internal carotid plexus, sympathetic fibers through the ciliary ganglia, and short ciliary nerves to the vasomotor effectors in the contralateral eye. Since parasympathetic fibers to the eye innervate ciliary and iris smooth muscle and have no vasomotor effect, it is unlikely they would play a part in causing histologic alteration. The finding that sympathectomy prevents the occurrence of the contralateral reaction is presumptive evidence that sympathetic impulses cause it.

Vasoconstriction as Cause of Contralateral Reaction: The histopathological changes in the contralateral eye could have resulted secondarily from vasomotor effects. The effect of vasoconstrictor fibers on intraocular blood vessels in rabbits is particularly strong, although mostly indirect. Stimulation of these fibers constricts the extensive amount of smooth muscle found in the orbit and eyelids. This causes exophthalamos, raises the intraocular pressure by compression of the globe, and produces stasis of blood in the eye by compression of veins at their outlet (Adler [21]). There is also good experimental evidence for effective vasoconstrictor fibers to the arterioles within the eye (Adler et al.[22]). Bárány [23] has shown that these are not involved in intraocular blood flow following changes in systemic blood pressure, but this does not exclude their possible role in reflexes resulting from antigen-antibody reactions. Experimentally produced intraocular arteriole spasm causes diminished blood flow through capillary beds throughout the eye. The local tissue anoxia soon causes the capillaries to dilate and to become more permeable (Adler [21]). These abnormal vasomotor impulses to the contralateral eye over a period of several days could cause secondarily the mild local hyperemia, edema, and round-cell infiltration.

Hypothesis of Neural Mediation: The following hypothetical explanation can now be made for the bilateral reactions. When the challenge dose of antigen is given intravenously it circulates to the sensitized eye and reacts with the local cellular antibodies with an intensity that is visible grossly. Undoubtedly these histopathological changes bring on the alarm reaction. The tissue injury is surely great enough to affect sensory receptors in the eye, and impulses are propa-

gated through the intact ophthalmic division of the fifth cranial nerve. The motor reactions of the central nervous system to this information are expressed through sympathetic fibers. The increased motor discharge of impulses through sympathetic pathways back to the eye causes vasoconstriction, increased intraocular pressure, and some pupil dilation. This alarm reaction decreases the blood flow and, therefore, the amount of injurious antigen passing through the capillary bed. This not only prevents more tissue destruction but also initiates tissue changes, namely, compensatory vasodilation, edema, and round-cell infiltration, which represent the beginning of the repair processes. To explain the vascular changes in the contralateral eye, we need only assume that, while the stimulation of sensory fibers is unilateral, the response is bilateral.

This bilateral reflex tempers and dampens the antigen-antibody reaction in the sensitized eye, disturbing normal tissue milieu but at least initiating the repair processes of the inflammatory response. It simply disturbs the normal tissue milieu unnecessarily in the contralateral eye, secondarily causing the milder histopathological changes found in the contralateral eye.

Contralateral Eye Compared to "Shock Organ" in Allergy: Some support for the hypothesis that an antigen-antibody reaction in one eye can cause a reflex inflammatory reaction in the other eye may be found in a comparison of events with the reflex expression of allergic reactions in specific "shock organs." There is justification in making such a comparison if the general opinion is accepted that the mechanism of allergy is essentially the same as the mechanism of the immune response (Taub[24]). In the case of specific food allergies, different expressions are known to occur in different "shock organs" in different people. For example, following the ingestion of a food allergen, one susceptible person may have dermatological symptoms, another may have neurological symptoms, another may have respiratory symptoms. This referred expression of an allergic reaction in the intestine to one or another different shock tissue is believed by Kuntz[20] and Urbach[19] to be a function of the limited distribution of autonomic fibers, usually parasympathetic, to specific shock organs. Whether the reaction in the shock tissue is purely reflex or whether the reaction is a result of autonomic disbalance in the shock organ which facilitates its sensitization to food allergens in the general circulation, a neural reflex mechanism is implicated involving visceral sensory fibers, central nervous system mediation, and a limited parasympathetic motor distribution to the shock tissue.

There is justification in comparing the contralateral eye reaction to the shock tissue reaction in an allergy if we consider the specific distribution of sympathetic fibers to the eye. In the case of the contralateral eye reaction, the specific distribution of sympathetic fibers could play the same role as the specific distribution of parasympathetic fibers to visceral shock organs. For while the distribution of sympathetic fibers is very diffuse to thoracic and abdominal organs, one organ receiving impulses from many different levels of the cord, the sympathetic distribution to the eye arises from a specific area of preganglionic cells in the second thoracic level of the spinal cord.[17]

Just as sympathectomy of the contralateral eye prevented the contralateral reaction, so also there are many reports of modification or elimination of shock organ allergic effects by surgical interruption of either sensory or motor arms of reflex pathways. Coca[25] reports many cases of dramatic relief from food allergies, following sympathectomy of large areas of the intestine by removal of celiac and lumbar sympathetic ganglia. The beneficial effect, he believes, is due to interruption of visceral sensory fibers from the intestine, which prevents sensory impulses from reaching the central nervous system that in turn find motor expression in various "shock organs." Similarly, symtomatic relief from respiratory allergies has often been achieved after the surgical interruption of either sensory or motor pathways to the bronchi.[19,26]

One cannot explain one enigma, the effects of sympathectomy on the bilateral rabbit eye reaction, with another enigma, the effect of sympathectomy on shock tissue reactions in human food and respiratory allergies. However, the modifying effects of sympathectomy in both cases supports the hypothesis that a neural mechanism involving a reflex arc exists. If the experimental results are explained with a neural mechanism involving central nervous system mediation, we have a ready explanation for the variations in the contralateral reactions in the control groups of rabbits. Central nervous system mediation brings in the possibility of innumerable other influences. With two rabbits of equal size and administration of equal sensitizing and shocking doses, it might take considerably more antigen-antibody reaction in the rabbit with a higher central excitatory state for this particular reflex to cause the contralateral reaction.

Sympathetic Ophthalmia Compared to Contralateral Reaction: Interest in the contralateral rabbit eye reaction grew out of an atempt to find in it an explanation for sympathetic ophthalmia. While there is a superficial resemblance in the two phenomena, the following comparisons will illustrate the many fundamental differences.

In the experimentally produced bilateral rabbit eye reactions, a known antigenic protein is injected intravitreously into the originally involved eye, specific circulating antibodies can be detected, and the contralateral reaction can be produced in 80% to 100% of the rabbits. In contrast, in sympathetic ophthalmia, there is trauma in the originally involved eye, but no known release of antigenic material other than uveal pigment is suspected in this trauma. In those cases where there has been traumatic release of uveal pigment to the blood, there are no detectable circulating antibodies. Indeed, sympathetic ophthalmia in humans is a relatively rare condition following uveal pigment release (Alvaro [15]). Again, no support for the uveal pigment factor in sympathetic ophthalmia can be derived from rabbit experiments. Schlaegel [28] found no difference between albino and pigmented rabbits in their ocular reactions to injection of horse serum and in the number of contralateral reactions.

The experimentally produced contralateral rabbit eye reaction can be elicited by a shocking dose of antigen 7 to 14 days after sensitization. Sympathetic ophthalmia, in contrast, occurs after an interval of several weeks or many months by an agent as yet unknown.

In the rabbit eye reaction, the histopathological picture in the contralateral eye is of a mild inflammatory nature, with moderate round-cell infiltration followed by complete recovery. In human sympathetic ophthalmia the histopathological picture in the sympathizing eye is of an intense infiltration of round cells, with complications that often produce blindness and loss of the eye. Schlaegel and Davis [6] were able to induce a histopathological picture in the rabbit eye resembling that of sympathetic ophthalmia, but it was done by conducting severe antigen-antibody reactions in eyes acutely sensitized by intraocular injection. In no case was a similar severer histopathological picture produced in a contralateral eye, where the reaction was always mild.

The experimental results of this paper are observations of the modifying effect of sympathectomy on the rabbit reaction. This and corollary evidence strongly suggest a neural mechanism is solely responsible for the contralateral reaction. Concerning the explanation of sympathetic ophthalmia, Alvaro [15] states that the assertion of Butler [29] perhaps still holds, namely, "We know literally nothing about the ultimate cause of the disease."

Clearly, the fundamental differences between the two phenomena provide absolutely no basis for suggesting that sympathectomy of the uninjured eye would prevent sympathetic ophthalmia. However, these differences do not preclude the possibility of some other neural mechanism in the etiology of sympathetic ophthalmia.

Promise of Fluorescent Microscopy: A complete explanation of why the contralateral eye reaction does not occur in a

sympathectomized rabbit eye has not been achieved in this investigation. Evidence has been cited that discounts sympathetic denervation affecting a possible antigen-antibody reaction in the contralateral eye. Little support is found for "elective sensitization" solely by a humeral mechanism. On the other hand, experimental evidence has been gathered and corollary facts have been assembled to support the explanation that the contralateral reaction is solely due to neural reflexes. Determination of whether or not antibodies are formed in the cells of the contralateral eye in "elective sensitization" is pertinent to a more complete explanation. In this regard, Witmer [30] has recently used the techniques of Coons, employing fluorescent microscopy to detect specific antibodies in sensitized eyes. This technique should be of use to determine if, upon injection of antigen into one rabbit eye, antibodies can be detected in the cells of the other eye. If antibodies are formed in the "electively sensitized" eye, it would support the explanation that sympathectomizing the eye can have a direct modifying effect on subsequent antigen-antibody reactions. If antibodies are not found in the "electively sensitized" eye, this would suport the explanation that "elective sensitization" is based essentially on a neural mechanism. It would also suggest that other hitherto unexplained tissue reactions in the eye may be referred manifestations of immune and allergic reactions elsewhere in the body.

Summary

A severe antigen-antibody reaction in a rabbit eye sensitized by injection and challenged intravenously may be accompanied by a mild contralateral eye reaction. This contralateral reaction has been attributed either to various immunological mechanisms involving "elective sensitization," to infectious agents, or to neural reflexes.

The finding of this investigation is that sympathetic denervation of the sensitized eye has no modifying effect on subsequent antigen-antibody reactions. However, sympathectomy of the contralateral eye will prevent the occurrence of the contralateral reaction.

Little support can be mustered for the explanation that sympathetic denervation of the contralateral eye modifies any subsequent antigen-antibody reaction. Greater support is available for the explanation that sympathetic denervation of the contralateral eye interrupts the reflex pathways responsible for the contralateral reaction.

The contralateral reaction in the rabbit differs in many fundamental ways from sympathetic ophthalmia. Findings applicable to one should not be expected to apply to the other.

A more complete explanation of the contralateral reaction in the rabbit awaits positive or negative determination of specific cellular antibodies by techniques employing fluorescent microscopy.

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Experimental Investigations on Uptake of Radioactive Phosphorus in Ocular Tumors

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As our clinical investigations relating to uptake of radioactive phosphorus (P^{32}) by neoplastic and inflammatory lesions within the eye have proceeded during the past few years, it has become increasingly desirable to build a firmer foundation for these empirical clinical observations by means of controlled experiments in animals. In planning these experiments, the principal purpose has been to elucidate the clinical observations and to evaluate more accurately the various technical factors which enter into these determinations. Our investigations have been based on a large body of work and knowledge already gained by others on the uptake of radioactive material, particularly radioactive phosphorus, by various types of malignant tissue and on the facts that have been learned about the metabolism of phosphorus in normal and malignant cells. In addition, we could avail ourselves of a tested method of producing tumors in the eye, i. e., the technique of transplantation of malignant tumors into the anterior chamber, and could make use of the data accumulated by other experimenters in selecting tumors for transplantation most suitable for our purpose. Our object throughout this experimental work has been to correlate the experimental results with the clinical findings, with the idea of placing this ophthalmologic diagnostic method on a firmer basis, to define the indications of this method more definitely, and perhaps to learn something more of the behavior of malignant lesions within the eye that would be of value from the therapeutic standpoint.

Our experiments were carried out in three phases: (1) comparison of uptake of radioactive phosphorus in normal ocular tissue and experimental eye tumors; (2) study of the effect of vascularity on the radioactive uptake in neoplastic, inflammatory, and normal ocular tissue, and (3) correlation of the uptake of radioactive phosphorus with cellular structure and study of the metabolism of phosphorus and its intracellular distribution.

I. Historical Background and General Considerations

Studies with Radioactive Isotopes on Metabolism of Malignant Tumors.—In his pioneer experiments, in 1923, Hevesy [1] used a naturally occurring radioisotope, lead ^{212} or thorium B, to study the transport of lead in plants. After the discovery of artificially induced radioactivity, by Joliot and Curie,[2] in 1934, the first biologic application was that by Chiewitz and Hevesy,[3] who used radioactive indicators in the study of phosphorus metabolism in rats. Hevesy has continued active work in this field of investigation. A statement that he made in 1948 is particularly pertinent to present tumor studies: "Early experiments have shown that labeled phosphatides can be detected in tissues shortly after administration of labeled phosphorus. The percentage of the dose administered present in the various

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organs of the rat as phosphatide (P^{32}) first increases and then declines after about 10 hours." [4]

Time-distribution studies of radioactive isotopes in various organs and metabolic evaluation by means of tissue fractionation by many investigators established the fact that metabolically active organs, such as the liver, spleen, and adrenals, take up larger quantities of radioactive material, particularly radioactive phosphorus, than less active tissues.

Forssberg and Jacobsson,[5] in 1945, studied the distribution of intravenously injected labeled phosphorus in rabbits with the Brown-Pearce carcinoma and found that metastases take up on an average as much P^{32} as the most active organs, e. g., liver, spleen, and suprarenal. When the uptake of P^{32} per gram of wet weight of metastases was compared with uptake per gram of less active tissue, such as pleura, diaphragm, and lymph nodes, uptake of the former was as much as five times greater. Increased rates of uptake of P^{32} were observed also by Jones and associates,[6] by Lawrence and associates,[7,8] and by Kenney.[9] Lawrence and his collaborators showed that retention of P^{32} per gram in various tissues of the leukemic mouse was greater than in the normal mouse and that there was also a marked selective uptake of P^{32} by lymph nodes invaded by lymphosarcoma. Scott [10] observed that lymphomatous tissue retained more radioactive phosphorus than normal tissue for 1 to 19 days after administration of labeled phosphorus.

Numerous attempts have been made to apply these experimental findings to diagnosis or treatment of human cancers. The short half-life and the limited power of penetration of the β-rays emitted by P^{32} make it unsuitable for treatment of internal cancers. It has become, however, an accepted and standardized method in treatment of polycythemia vera, and some success has been reported in certain types of skin cancer.[11,12]

Moore and his associates [13,15] carried out clinical and experimental studies on the diagnosis of central nervous system tumors, using the γ-ray emitter, diiodofluorescein containing I^{131}. First trial of P^{32} as a possible diagnostic aid in malignant disease was made by Low-Beer,[16] in 1946, and later by McCorkle and associates,[17] in measurements of uptake of radioactive phosphorus in breast tumors.

Selberstone and his co-workers [18,19] and Sweet [20] reported the use of radioactive phosphorus in the localization of brain tumors. Dunphy and Selberstone [21] (1950) reported the distribution of radioactive phosphorus, as determined by in vitro counts, in ocular tissues removed after intravenous injection of P^{32}.

Application of the principle of differential uptake of radioactive phosphorus by malignant tissue, by in vivo counts, as a means of diagnosis in intraocular tumors began with the work of Thomas and his associates [22] in 1952. Subsequent studies have been reported by Trevor-Roper et al.,[23] Towne,[24] Krohmer et al.,[25] Dunphy et al.,[26] Thomas et al.,[27] Bettman and Fellows,[28] Eisenberg and associates,[29] and Dunphy et al.[30]

Choice of Isotope.—Since the purpose of this experimental investigation is to collect data from animals that can be compared with data obtained in clinical cases of intraocular lesions after injection of radioactive phosphorus, this substance would logically be the isotope used. Nevertheless, the many advantages of P^{32}, which made it the material of choice in developing the clinical test for intraocular lesions, should be kept in mind. In the first place, the universal distribution of phosphorus in cell nucleoprotein makes this element an ideal one for study in a wide range of metabolic investigations. Nucleoproteins are known to be synthesized more rapidly by tumor cells than by normal tissue, resulting in a measurable differential uptake of radioactively labeled phosphorus. Furthermore, the chemical toxicity of P^{32} is low, as has been proved by its extensive use in treatment of polycythemia vera. P^{32} has a half-life of 14.3 days, which is sufficiently long to allow for shipment, standardization, and preparation of doses without prohibitive

loss of material and yet is short enough to be compatible with the time scale of the experiment. P^{32} emits only β-rays which have a very short range in tissue, i. e., about 3 to 4 mm.; hence, radiation hazard is minimized. The extensive documentation of the properties and biologic and physiologic effects of radioactive phosphorus is another advantage, since it was the first isotope to be used experimentally and had been the one most widely employed in all types of metabolic research subsequently. As a final practical advantage, P^{32} is easily available in standardized form. It is prepared by a separation process from the fission products of the Oak Ridge reactor and is colorless, odorless, and tasteless. It is received as a solution of disodium hydrogen phosphate or phosphoric acid, almost completely carrier-free and with a specific activity of 5 to 7 mc. per cubic centimeter. It is standardized by the method established by the Bureau of Standards.

From the foregoing, it is evident that radioactive phosphorus (P^{32}) fulfills admirably (for our purposes) the criteria laid down by Heidelberger [31] in choosing a tracer for a particular experimental study: "The isotope must behave chemically and biologically in a fashion identical to that of the natural element. The isotope must be present in sufficiently high concentration to withstand the dilution that is encountered in the experiment. The label must be retained throughout the experiment. There must be no biological effect caused by the toxicity of the tracer itself. The half-life of the radioactive isotope should be long enough for the experiment. The labeled atom should be an isotope of the element of actual interest."

Evaluation of Radioactive Counts from Tumor Tissue.—Studies on the uptake or turnover rate of a radioactive isotope in a given tissue yield measurements of radioactivity representing a combination of several different effects: (1) the contribution to the counting rate due to radiation from the tissue under study, (2) radioactivity due to presence of the isotope in some tissue or tissues other than the one being studied, and (3) background count due to cosmic rays and to radioactive material in the general vicinity of the counter.

Dunphy and associates,[26] in their work on localization of brain tumors, believed that the much greater concentration of P^{32} in these tumors was due rather to the extreme sluggishness of uptake of normal cerebrum than to a very rapid uptake by the tumor. Some active normal tissues, such as liver, spleen, and bone marrow, take up phosphorus more rapidly than many malignant gliomas, and skeletal muscle takes it up at rates similar to those seen in gliomas. Hence, these authors concluded that the diagnostic test with P^{32} is likely to be applicable only to tumors which grow in a bed of metabolically slow-moving normal tissue. The eyeball qualifies in this respect because a large volume of the globe is taken up by the "metabolically torpid" sclera, aqueous, vitreous, cornea, and lens. The more active choroid and retina are metabolically active and hence have high rates of uptake of radioactivity, but these are small structures, representing only a small proportion of the total eye volume.

In Vivo Measurements.—The accuracy of in vivo measurements of uptake of a radioactive substance depends upon several factors, one of which is time. The rate of change after injection of the isotope is measured in abnormal fields and compared to corresponding measurements in normal areas. To reduce experimental error and to increase accuracy, the counting must be carefully timed. Since counter measurements are subject to statistical errors, as much time as possible should be used to collect useful experimental data. In planning in vivo experiments, it is necessary to estimate the time scale accurately, to have some idea of the probable sources of background radiation, and to evaluate accurately the limitations and capabilities of the avail able equipment. The main features of the experiment can then be planned to yield the maximum of useful information when a

given quantity of radioactive material is used.

Veall [32] recommends attention to the following factors in determining accuracy of in vivo measurements: (1) reduction of background radiation to a value small compared with the intensity of radiation from the region being studied, (2) proper design of equipment so that it can be set up and manipulated without waste of time, (3) automatic recording of data, and (4) maximal intrinsic efficiency of the radiation-detecting equipment.

Specially Designed Eye Counter Probes. In our first clinical experiments involving radioactive counts over the eye, it became evident that special probes with Geiger counters were desirable or necessary, especially in taking readings over posterior portions of the eye. The result was the development of the clinical end-window and angle-window probes, which have been used successfully on the eyes of patients. These clinical counter probes were used in our first experiments on animal eyes, but both the end-window and the angle-type counters proved to be somewhat large for obtaining satisfactory readings over the eyes of small animals, especially in the guinea pig experiments. It was felt that a smaller Geiger counter was desirable, even for the rabbit eye, which has a much larger anterior segment. Hence, a much smaller mica-window Geiger-counter tube has been developed [33] which has greatly facilitated the in vivo counting procedure in these animal experiments.

Transplantation Experiments.—A very prominent feature of cancer research during the past decade has been transplantation of neoplasms of all types—into new sites in the same host, into littermates, into animals of the same species, and, finally, heterologous transplantation from one species to another. Some experiments have been done also in transplanting neoplasms from one zoologic class to another, e. g., animal tumors into chickens. All types of primary animal tumors have been transplanted homologously or heterologously, and in the past few years many human malignant lesions have been successfully transplanted into laboratory animals. In these studies, host factors and factors intrinsic to the neoplasm have been separately and collectively studied, methods have been standardized, and a great body of data has accumulated as to the transplantability of various types of new growths and the conditions that govern this process.

Most pertinent for our purposes have been the studies of Green and his collaborators at Yale, who developed the method of intraocular transplantation and have studied the behavior of a great many animal and human tumors when transferred in this way.[34-38] Their work, as well as that by others, has shown that a consistent relationship exists between the degree of malignancy and the transplantability of a tumor. As Greene [38] explains it, a malignant tumor remains in a state of "dependency" on factors in the host to a certain stage of its development, when it becomes "autonomous" and can continue to grow and propagate itself independent of environmental conditions in the host. Autonomy and ability to metastasize, as well as transplantability, apparently are manifestations of the same phenomenon. Towbin [39,40] designates this the "growth momentum"; this governs the rate of enlargement, infiltration, and metastasis, which indicate the degree of malignancy of the tumor. Heterologous transplantability is especially dependent on acceleration of growth momentum. During early stages of neoplasia, heterologous transplantation frequently fails because growth momentum is low. Later, when growth momentum has increased and infiltration and metastasis have occurred, heterologous transfer of the tumor is more often successful.

In the experiments to be described here, neoplasms grown in the anterior chambers of the animal eyes were primary animal tumors. The Murphy lymphosarcoma, the Brown-Pearce carcinoma, and the S-91 mouse melanoma offered the most suitable material. A strain of glioblastoma was also induced by implantation of 3-methylcholan-

threne into the cerebrum of white rats. When transferred to the anterior chamber of the eye, 75% of the transplants grew satisfactorily.

Production of Experimental Ocular Tumors.—Preparation of Transplant: Immediately after the tumor specimen is obtained, it is refrigerated in a sterile Petri dish. Portions for transplantation are selected somewhat away from the surface, well into the cellular portion of the neoplasm. A portion is subjected to microscopic histologic study before transplantation.

Technique of Transplantation: Introduction of tumor tissue into the anterior chamber of animals' eyes is carried out essentially according to the technique developed by Greene.[34] Animals were anesthetized with pentobarbital (Nembutal) administered intraperitoneally in the guinea pigs and rats and intravenously (into the ear vein) in rabbits. The eye was fixed, and a small keratome was introduced into the anterior chamber at the 6 o'clock position. This portion was chosen, since the tumor can best be observed growing at the 12 o'clock position. If the tumor was of firm consistency, it was introduced into the anterior chamber with a small lens forceps, and if the consistency was spongy, a trocar was inserted through the wound and the injection was made with a syringe. Sterile conditions were maintained, an important feature in success of the procedure. After its insertion, the tissue fragment was massaged into the anterior chamber angle at the 12 o'clock position by pressure on the corneal surface with an iris spatula.

Observation: Animals were observed regularly in order to follow the progress of neoplastic growth in the anterior chamber. Anterior chamber tumors evolve through three stages[39]: suspension, while the fragments lie loosely in the anterior chamber; nidation, when the fragment organizes and becomes attached to the iris, with vascularization, and growth, when the tumor increases in size by multiplication of cells and increased vascularity. In our experiments, the Murphy lymphosarcoma was transferred successfully to both guinea pigs and rabbits. The Brown-Pearce carcinoma (supplied by Dr. Harry Green) is advantageous for experimental work because of its rapid and easy growth. In the rabbit eye, retransplants from this tumor afforded a ready supply of neoplastic material (Fig. 1). The S-91 melanoma grows slowly but also provides practically 100% yield of tumor tissue for investigation.

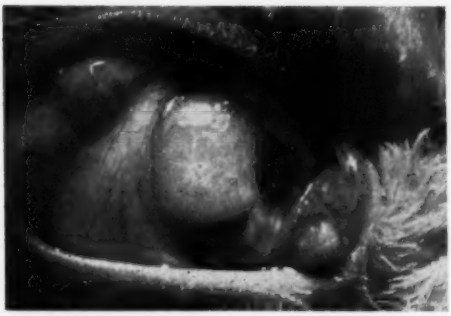

Fig. 1.—Brown-Pearce carcinoma in the anterior chamber of a rabbit eye.

Growth of tumor in the anterior chamber is satisfactory for in vivo determinations of radiophosphorus uptake when tumors are about 5 to 15 mm. in diameter. Most of these tumors grew in the anterior chamber, but in some guinea pig eyes, tumor fragments became lodged behind the iris and grew in the region of the ciliary body and posterior segment.

II. Comparison of Uptake of Radioactive Phosphorus in Neoplastic and Normal Ocular Tissue

Experiments were carried out to determine (1) the levels of radioactivity in malignant and normal tissue as a function of time or the rate of uptake of radioactive phosphorus by these various tissues, (2) the distribution of radioactivity in the neoplastic growth compared to normal tissue at a given time after injection of P^{32}, and (3) the relationship between radioactive uptake and neoplastic growth, by comparison of in vivo radioactive counts with histologic studies.

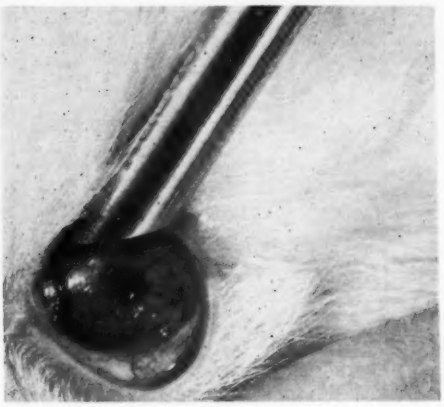

Fig. 2.—Geiger-counter probe over anterior chamber of rabbit eye containing Brown-Pearce carcinoma.

Methods.—In Vivo Counting Procedure: The animal was anesthetized by injection of thiopental (Pentothal) sodium. After preliminary checking of the counting apparatus, counts were begun immediately after injection of the P^{32} (0.05μc. per gram of body weight) by placing the special small mica-window Geiger-counter tube in direct contact with the eye, over the neoplasm in the anterior chamber (Fig. 2). (Histologic section of the eye shown in Figure 2 is reproduced in Figure 3.) Measurement of radioactivity was continued for one-half to one minute in this location, and the count was recorded. The counter was then moved to an uninvolved portion of the eye as far

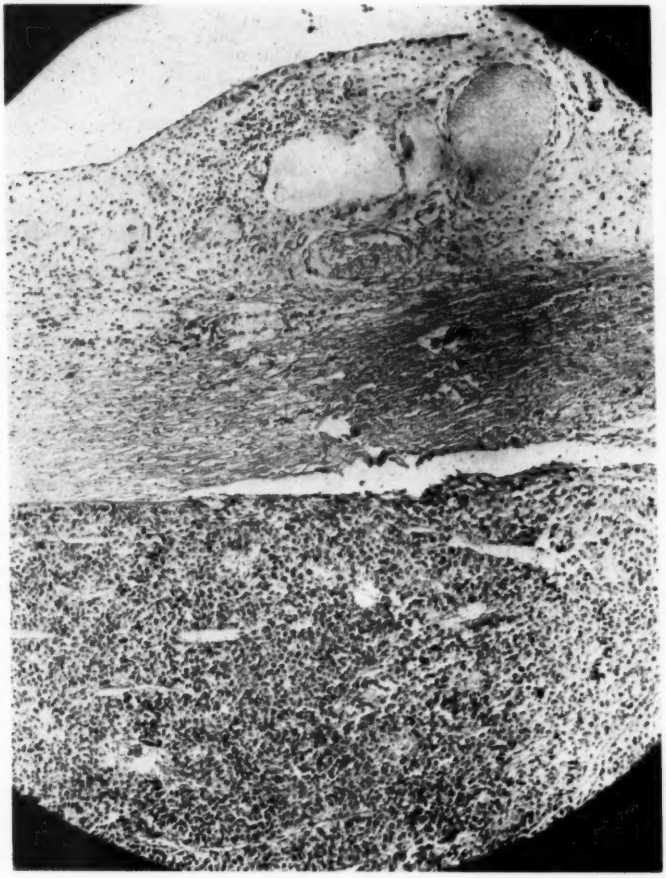

Fig. 3.—Histologic section of eye containing Brown-Pearce carcinoma in the anterior chamber; reduced about 10% from mag. $\times$ 90.

removed from the tumor site as possible, or to the other normal eye, and a similar count was taken. Counts were measured at specific intervals, usually within a minute after injection, and continued until some stability was obtained. After the peak of radioactivity was reached (usually within 15 to 20 minutes after injection), the time interval between counts was lengthened. Comparative counts were then repeated at intervals up to three days after injection of the radioactive phosphorus. From these results, curves were plotted with the time interval as the abscissa and radioactive counts per minute as the ordinate. The variation in counts for tumor and for normal tissue, as a function of time, was plotted on ordinary graph paper, and the ratio of tumor-tissue to normal-tissue counts, determined at various times, was calculated.

In Vitro Counting Procedure: The eye was enucleated and immediately sectioned through the tumor. One-half of the specimen was used for preparation of histologic sections. From the other half of the eye, samples of tumor, sclera, or uveal tract were obtained for assay of radioactivity. Such counts must be made on fresh specimens, for if tissues are allowed to remain in fixative for any length of time, there is loss of radioactivity by leeching.

The aliquots were weighed wet (wet weights of tumors ranged from 0.3 to 0.8 gm.) and placed in porcelain counting dishes, dissolved in concentrated nitric acid, and then dried, thus yielding thin flat specimens. After drying, samples were counted under identical conditions, with use of a conventional laboratory Geiger counter. Results were recorded, after correction for background radiation, in counts per minute per gram of sample. Ratio of these values for tumor to normal tissue was then calculated.

Tissue Geometry: In many of the curves illustrated below there is a great variation in amount of radioactivity accumulated in different tumors and in various areas of the same tumor. This difference in radioactive

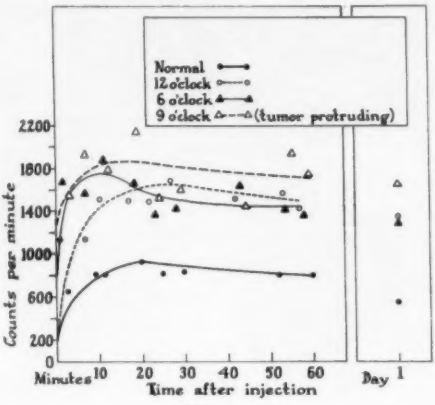

Figure 4

accumulation is due in part to a factor of tissue geometry.

Relative counting efficiencies of various geometric placements of the counter over the tumor-bearing eye (Brown-Pearce carcinoma in the rabbit eye) show the maximum uptake at the site of tumor protrusion, with approximately 20% decrease at a 90-degree radial variation in both directions (Fig. 4). All three sites, however, have exhibited an increase of 100% or more over the counting rate of the normal eye, indicating that in this instance either the tumor is sufficiently large or the uptake is so widespread that requirements of exact geometric placement are not so rigid as in smaller eyes with other types of tumors. It is interesting that this differential is maintained even to 24 hours after injection and that at that time the maximum recording site may still be identified with its 20% increase over adjacent areas.

Since the P^{32} diffuses readily from the vascular system into tissue space, a continuously changing tissue geometry is presented to the Geiger counter. Thus, the curve presenting the in vivo P^{32} counting rate shows a continually changing ratio between the P^{32} in the vascular system and that in tissue space. As the P^{32} diffuses into the tissue, the geometric efficiency increases because of the inverse square law, and so a small diffusion into tissue space may

TABLE 1.—*In Vitro Radioactive Counts After P^{32} Injection in Transplanted Ocular Tumors*

Animal	Tumor	Specimen	Counts/ Min.	Counts/ Min/ Gm. *	Ratios	Histologic Findings
Rabbit 94	Lymphosarcoma	Wet			Wet	Mass of lymphoid cells in edematous richly vascularized connective tissue; no necrosis; no radiation effect detected
		Tumor & cornea	82	5,900	Tumor & cornea: cornea, 1.725:1	
		Cornea	27	3,420		
		Iris	50	6,330	Tumor: iris, 0.933:1	
		Sclera	67	4,930	Tumor: sclera, 1.198:1	
		Dry			Dry	
		Tumor	82	26,400	Tumor: cornea, 2.135:1	
		Cornea	27	12,380		
		Iris	50	27,800	Tumor: iris, 0.950:1	
		Sclera	67	11,980	Tumor: sclera, 2.2:1	
Rabbit 23	Lymphosarcoma	Wet			Wet	Richly vascularized collagenous connective tissue with collections of immature lymphoid cells
		Tumor	88.5	11,970	Tumor: cornea, 2.98:1	
		Cornea	24	4,000		
		Sclera	27	2,500	Tumor: sclera, 4.75:1	
		Iris	46.3	9,650	Tumor: iris, 1.24:1	
		Dry			Dry	
		Tumor	88.5	55,400	Tumor: cornea, 4.6:1	
		Cornea	24	12,000		
		Sclera	27	9,650	Tumor: sclera, 5.74:1	
		Iris	46.3	35,600	Tumor: iris, 1.56:1	
Rabbit 9	Lymphosarcoma	Wet			Wet	Immature lymphoid cells in several masses of discrete lymphocytes; in anterior chamber, attachment of neoplasm to ciliary body iris; some purulent inflammation
		Tumor	60.5	35,600	Tumor: cornea, 2.41:1	
		Cornea	21.8	14,530		
		Iris	78.4	22,400	Tumor: iris, 1.56:1	
		Sclera	25.3	7,480	Tumor: sclera, 4.78:1	
		Dry			Dry	
		Tumor	60.5	50,400	Tumor: cornea, 3.00:1	
		Cornea	21.8	16,770		
		Iris	78.4	56,000	Tumor: iris, 0.90:1	
		Sclera	25.3	9,370	Tumor: sclera, 5.38:1	
Guinea pig 2	Lymphosarcoma	Wet			Wet	Tissue mass in anterior chambr partly composed of discretely arranged groups of immature lymphoid cells with vesiculated nuclei; some inflammatory changes; occasional mitosis
		Cornea	113	51,400	Tumor: cornea, 1.155:1	
		Tumor	344	59,400		
		Sclera (near tumor)	338	31,700	Tumor:sclera (near tumor), 1.68:1	
		Sclera (far from tumor)	304	19,600	Tumor: sclera (far from tumor), 3.03:1	
		Dry			Dry	
		Cornea	113	59,500	Tumor: cornea, 1.09:1	
		Tumor	344	65,000		
		Sclera (near tumor)	338	51,200	Tumor: sclera (near tumor), 1.27:1	
		Sclera (far from tumor)	304	27,200	Tumor:sclera (far from tumor), 2.39:1	

TABLE 1.—*In Vitro Radioactive Counts After P^{32} Injection in Transplanted Ocular Tumors*—Continued

Animal	Tumor	Specimen	Counts/ Min.	Counts/ Min/ Gm. *	Ratios	Histologic Findings
Guinea pig 5	Lymphosarcoma	Wet			Wet	Viable sarcoma
		Tumor	370	21,500	Tumor: sclera, 1.7:1	
		Tumor (posterior)	154	17,100		
		Tumor (necrotic center)	576	20,000		
		Sclera (invaded by tumor)	63	12,600		
		Dry			Dry	
		Tumor	370	75,500	Tumor: sclera, 3.0:1	
		Tumor (posterior)	154	55,000		
		Tumor (necrotic center)	576	75,000		
		Sclera (invaded by tumor)	63	25,200		
Rabbit 26	Brown-Pearce carcinoma	Dry			Dry	
		Tumor	2,546	34,220	Tumor: sclera, 6.1:1	
		Sclera	393	5,630		
Rabbit 49	Brown-Pearce carcinoma	Dry			Dry	Tumor occupies anterior chamber & consists of diffuse sheets & masses with some regions of hemorrhage & necrosis; nuclei in general are large & vesicular; cells show active mitosis
		Tumor	1,259	6,728	Tumor: sclera, 2.41:1	
		Sclera	380	2,792		
Guinea Pig 42	Human retinoblastoma	Dry			Dry	
		Tumor		1,014	Tumor: normal eye, 1.34:1	
				1,197		
		Normal eye		805		
				847		
Guinea Pig 31	Human cylindroma of lung	Dry			Dry	
		Tumor		25,982	Tumor: normal eye, 1.59:1	
		Normal eye		16,352		
Guinea Pig 49	Human scirrhous carcinoma of breast	Wet			Wet	
		Tumor	359	51,286	Tumor: cornea (tumor eye), 1.02:1	
		Cornea (tumor eye)	513	49,800		
		Sclera & cornea (tumor eye)	48	811		
		Cornea (normal eye)	184	14,600	Tumor: cornea (normal eye), 3.46:1	
		Sclera & choroid (normal eye)	1,121	17,200		
		Dry			Dry	
		Tumor	359	179,500	Tumor: cornea (tumor eye), 1.5:1	
		Cornea (tumor eye)	513	121,700		
		Sclera & choroid (tumor eye)	48	5,720		
		Cornea (normal eye)	184	40,900	Tumor: cornea (normal eye), 4.0:1	
		Sclera & choroid (normal eye)	1,121	11,000	Tumor: sclera, 1.6:1	

* All counts were made with dry samples, but counts/min/gm. were determined for both wet and dry weights.

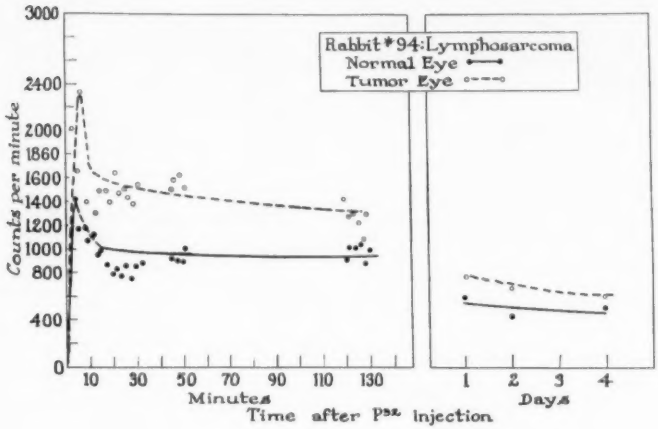

Fig. 5. — Pattern of radiophosphorus uptake in lymphosarcoma in rabbit eye.

represent a higher counting rate than a larger concentration from the deeper vascular bed. Difference in absolute levels may therefore represent either a larger concentration or merely a more favorable tissue geometry when the P^{32} has reached a closer proximity to the counter.

Results.—Comparative in vivo and in vitro measurements of radioactivity were made in the eye tumor and in normal ocular tissues in 25 rabbits and 25 guinea pigs with transplanted tumors of various types, and in vivo counts were made on many additional animals. Table 1 contains the data on 10 experiments, which are typical of the entire series, and the curves in Figures 5 to 9 illustrate the various patterns of uptake response. In every tumor studied, the in vivo measurements indicated a more rapid uptake (1.5 to 6 times greater) of radioactive phosphorus by the tumor than by normal uninvaded eye tissue. These results were confirmed by in vitro measurements of both the wet and the ashed, or dry, fragments, again with values 1.5 to 6 or 7 times the average uptake per gram of normal tissue. In general, curves of counts per minute (corrected for physical decay of P^{32}) plotted against time of observation showed an initial rapid rise, reaching a maximum within 10 to 12 minutes after injection, and then a decline, perhaps rapid at first, followed by a slow decline

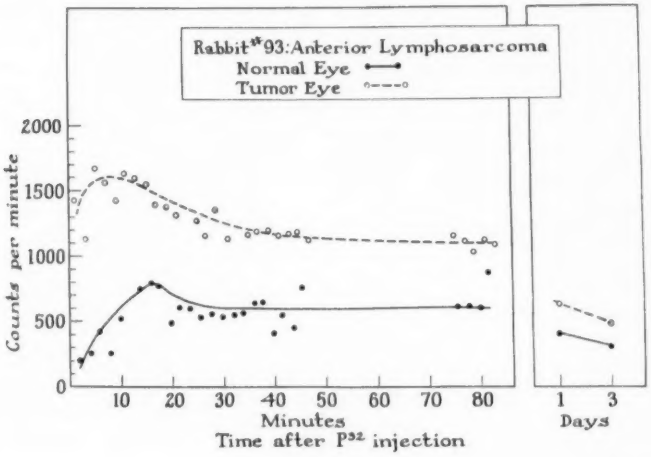

Fig. 6. — Pattern of radiophosphorus uptake in lymphosarcoma in rabbit eye.

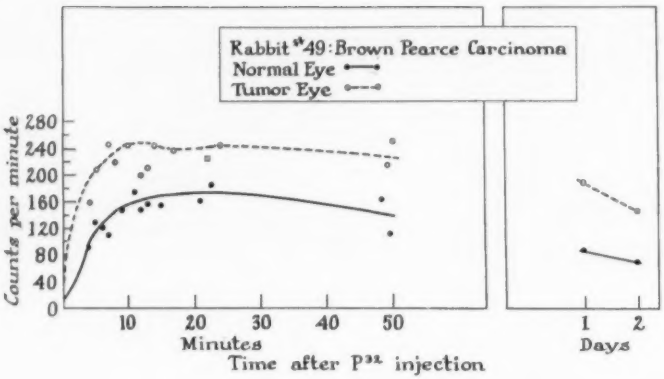

Fig. 7. — Pattern of radiophosphorus uptake in Brown-Pearce carcinoma in rabbit eye.

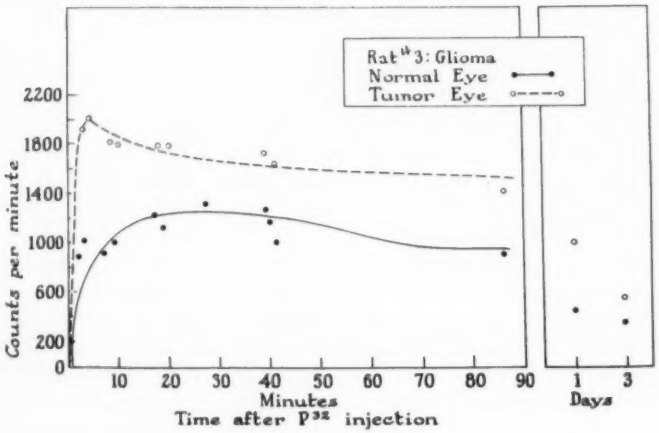

Fig. 8. — Pattern of radiophosphorus uptake in glioblastoma in rat eye.

extending over several days. During the initial rise in radioactivity immediately after injection, the blood levels of P^{32} also were high. It appears likely that during these first 10 to 12 minutes, the high counts are dependent largely upon vascularity of the tissue concerned. However, after the injected radioactive phosphorus becomes mixed with the phosphorus of the body and the metabolic processes begin to establish equilibrium in phosphorus exchange, the curves of uptake decline and follow a gradual pattern of decay.

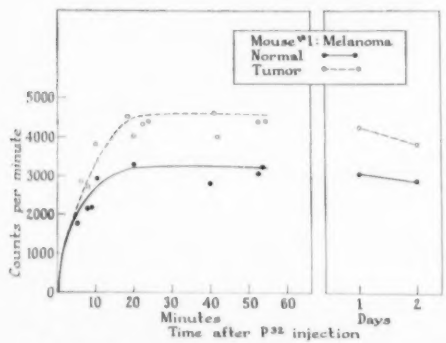

Fig. 9.—Pattern of radiophosphorus uptake in melanoma in mouse eye.

In Rabbit 94 (Fig. 5), with transplanted lymphosarcoma in one eye, the curve in both eyes displayed almost identical patterns, with initial rapid uptake and gradual decay, but radioactive counts throughout were significantly higher in the eye containing the tumor. In Rabbit 93 (Fig. 6), the curve is somewhat different, but the general ratio between normal and tumor eye is similar.

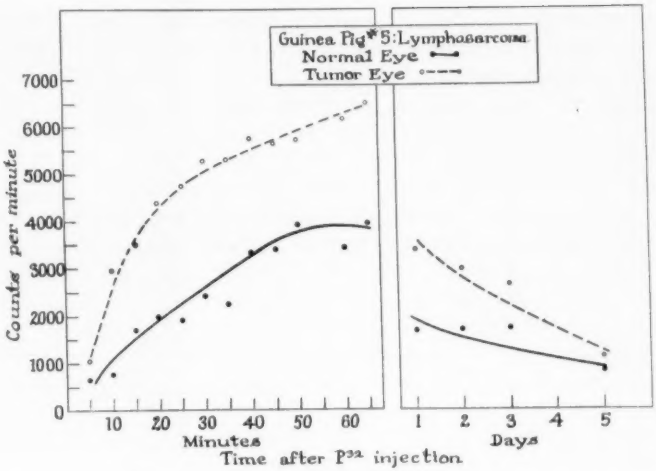

Fig. 10. — Pattern of radiophosphorus uptake in lymphosarcoma in guinea pig eye.

The more gradual rise in counts of the normal eye than of the eye with lymphosarcoma may possibly be explained by the fact that excessive vascularity accompanying the neoplastic growth may have increased the initial uptake of radiophosphorus in the cancerous eye. This tumor was situated well into the base of the iris and ciliary body.

In rabbits with anterior chamber growths of the Brown-Pearce carcinoma (Fig. 7), the pattern of radioactive phosphorus uptake was similar. This was also the case with the rat glioblastoma (Fig. 8) and the S-91 melanoma (Fig. 9). There was a rapid rise in both tumor and normal curves, with the tumor reaching a higher level and maintaining this differential throughout the period of decay.

In Guinea Pig 5 (Fig. 10), the slow increase in P^{32} uptake by the tumors and in the normal eye tissue is explained by the fact that these animals received the radioactive phosphorus intraperitoneally; this resulted in slower absorption by the tissues. The high count in Guinea Pig 5 (Fig. 10) at the end of 24 hours was at-

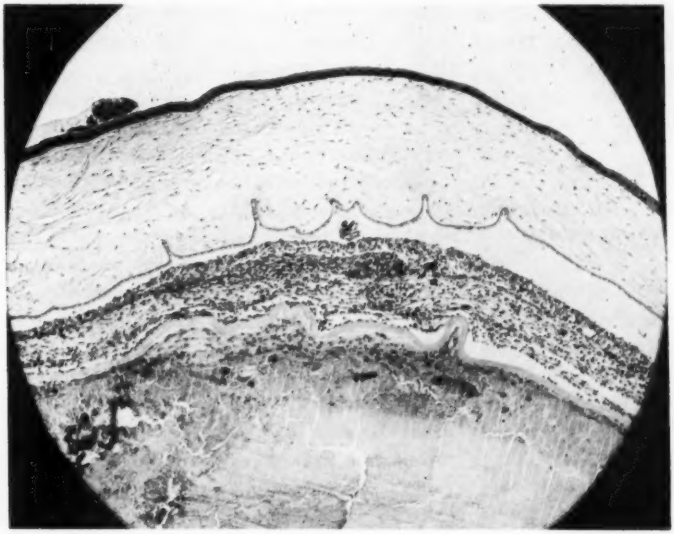

Fig. 11. — Histologic section of eye, showing tumor situated well into the base of the iris and anterior chamber.

tributable to an iritis initiated by trauma and to the fact that equilibrium in phosphorus exchange had not yet been attained. Later, the curve assumed the normal pattern. On histologic section, this tumor was found to be situated well into the base of the iris and anterior chamber (Fig. 11).

In the in vitro measurements, counts on ashed specimens were a little higher than the wet measurements, but in the majority of experiments both showed significantly higher radioactivity in the tumorous portion of the eye. In two tumorous eyes examined (Rabbits 94 and 9) the radioactive count of tumor tissue was less than in the iris. The presumed explanation is that the phosphorus turnover of this uveal tissue is high and simulates that of rapidly growing or rapidly dividing cells. In vitro counts in Guinea Pig 2 (Table 1) showed a high count for the cornea, with consequent reduction in ratio of tumor count to cornea count. This tumor had completely filled the anterior chamber, and neoplastic cells had already infiltrated the cornea.

These experimental results appear to follow the same pattern established by differential counts over tumors and other lesions in clinical studies on patients' eyes. Each tumor appears to possess a characteristic phosphatide turnover, independent of pathologic type and independent of phosphatide metabolism in the host. Maximal deposition of phosphatides (P^{32}) in neoplastic tissues may vary from 10 to 50 hours, as revealed in both in vivo and in vitro studies.

III. Effect of Vascularity on Radioactive Uptake in Neoplastic, Inflammatory, and Normal Ocular Tissue

The effect of vascularity upon the uptake of P^{32} by neoplastic, as compared to normal, tissue has been the subject of considerable speculation. Obviously, with greater vascular supply the tissue is exposed to more radioactive material and also has a greater capillary area for diffusion of the phosphate ion across the membrane into the tissue itself. Either of these mechanisms may result in higher deposition. In curves of radioactive uptake, the initial rapid rise, or that occurring during the first five minutes of observation, is thought generally to be due to vascularity.

Hence, this second phase of the study of uptake of radioactive phosphorus in experimental tumors deals with this problem by comparing the uptake of P^{32} by malignant tissue with that by inflammatory ocular tissue and by studying the influence of vascularity upon the uptake of radioactive phosphorus in normal, inflammatory, and neoplastic tissues.

Three sets of experiments were carried out:

1. Inflammatory corneal lesions were produced, and uptake studies were carried out over the lesions and simultaneously over normal ocular tissue; the pattern of accumulation was plotted against the element of time.

2. Perfusion studies were performed on eyes containing anterior chamber neoplasms. Comparative radioactive counts were made over both the neoplasm and the normal ocular tissue.

3. Comparative measurements of the uptake of P^{32} and iodinated (I^{131}) serum albumin were made over tumor tissue, normal tissue, and blood. This tagged (I^{131}) plasma was primarily distributed in the vascular system.

1. *Vascularized Corneal Inflammatory Lesions.*—Vascularized corneal inflammatory lesions induced on the external surface of the eye were used for comparative measurements of radioactive uptake in order to observe the effect of vascularity upon the uptake of radioactive material in general. The eye of the albino rabbit was anesthetized with 0.5% tetracaine (Pontocaine). A cotton applicator dipped in 10 M sodium hydroxide was applied to the cornea at four- to five-day intervals; this produced a granulomatous lesion which was somewhat raised and heavily vascularized (Fig. 12). After this lesion had become well established, radioactive uptake determinations

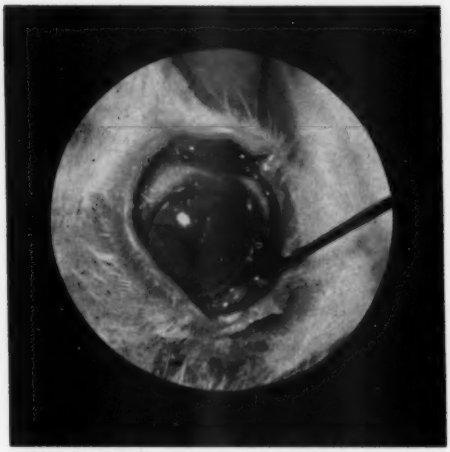

Fig. 12.—Inflammatory lesion of the cornea of a rabbit.

were made by the same in vivo counting procedure used in the first phase of the study. Similar graphs were made for variation in counts for the inflammatory lesion and for normal tissue as a function of time.

Results.—Comparative measurements in vivo of radioactivity over the inflammatory lesion and over normal tissue made in five rabbits were very consistent in every case. Figure 13 illustrates the typical pattern of uptake response. There is an initial rapid rise in radioactivity immediately after injection of the P^{32}, reaching a maximum in approximately 5 minutes, with a general decline thereafter, reaching the normal uptake curve in about 20 minutes. Afterward, the two radioactive levels run a parallel course throughout the two-day study. The high counts during the first five minutes after injection of radioactive phosphorus are presumed to depend largely upon vascularity present in the tissue concerned. This appears to be the case in this group of experiments, for the lesions over which counts were made were richly vascularized and contained only inflammatory tissue. The injected radioactive phosphorus rapidly reached an equilibrium with that already in the tissues, and the pattern of radioactive accumulation became similar in both the normal and the inflamed tissues. This apparently demonstrates that the influence of vascularity upon the radioactive accumulation after the injection of P^{32} is evident during the first 20 minutes and that the factor of vascularity accounts for the rapid initial and short-lasting rise in radioactive accumulation.

2. *Perfusion Studies.* — Rabbits with Brown-Pearce carcinomas occupying about one-third of the anterior chambers of both eyes were used for perfusion experiments. One of the terminal branches of the external carotid in the rabbit, namely, the internal maxillary artery, passes to the orbit through the anterior sphenoidal foramen in the root of the lateral lamina of the pterygoid process, giving off the inferior alveolar artery to the mandible and the inferior ophthalmic artery. The latter passes upward and forward on the medial wall of the orbit

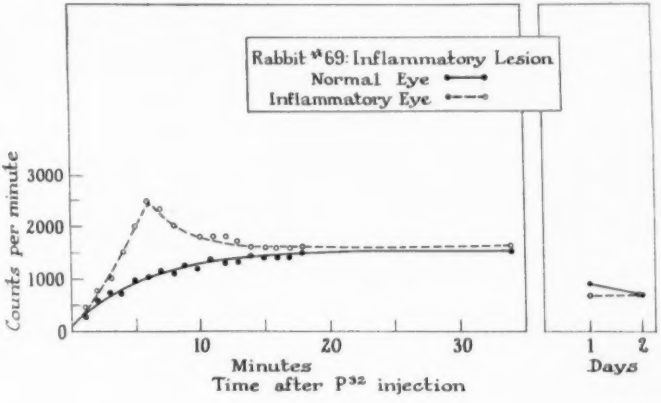

Fig. 13. — Pattern of radiophosphorus uptake in normal rabbit-eye tissue and in inflammatory ocular lesion.

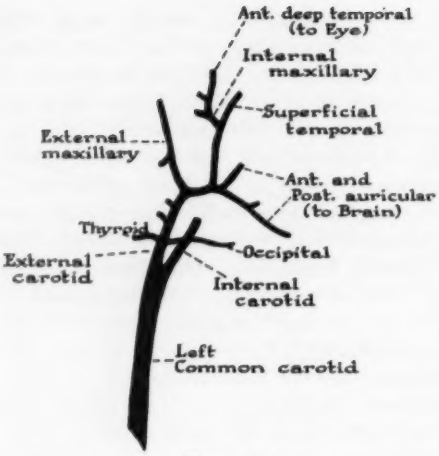

Figure 14

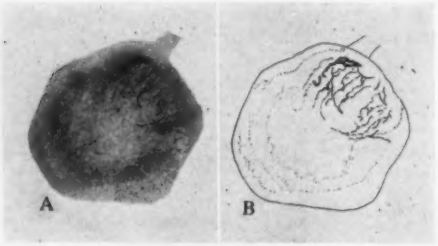

Figure 15

Fig. 14.—Branches of the common carotid artery of the rabbit.

Fig. 15.—*A*, radiograph of rabbit eye containing anterior-chamber Brown-Pearce carcinoma, given injection with radiopaque material (barium sulfate-gelatin mixture), showing vascular pattern outlined. *B*, drawing of outlined vascular pattern shown in *A*.

to the structures of the eye[41] (Fig. 14). The vascular pattern in the anterior chamber neoplasms was outlined by injecting barium solution into the perfusion system. Figure 15*A* shows the fine interlacing vessels containing radiopaque material and Figure 15*B* an associated drawing of this vascular pattern.

Two methods of perfusion were considered: (1) use of a buffered saline solution and (2) perfusion of the animal after injections of red cells tagged with Cr^{51} into the blood stream. Red blood cells of the rabbit were found to retain 86% of their radioactivity after the three washings prescribed. Preliminary experiments showed that loss of radioactivity is approximately the same, regardless of the method employed (Fig. 16). This dictated choice of the simpler method, i. e., perfusion with a balanced salt solution of the animal eye in which the neoplasm was situated.

The eye chosen for perfusion was usually the one with the smaller amount of hemorrhage and the one showing the more satisfactory vascular pattern. Perfusion was begun at intervals on the uptake curve of ½ hour, 1 hour, and 24 hours after injection of radioactive phosphorus. These intervals were chosen to correspond to the

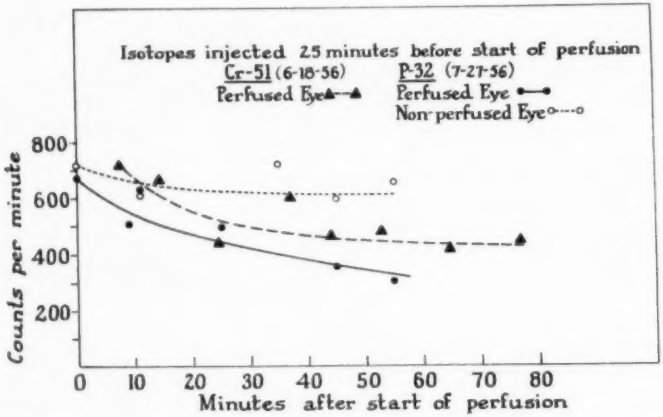

Fig. 16.—Perfusion of normal rabbit eyes following injections of red cells tagged with Cr^{51} into the blood stream.

readings made clinically in cases of suspected intraocular tumor. The rapidity with which the P^{32} became bound in the tissues could then be judged from the effect that perfusion of the tumor had upon the measured amount of radioactivity.

The animal was anesthetized with pentobarbital sodium containing 0.2 ml. of heparin (to prevent coagulation in the perfusion circuit). After a preliminary check of the counting apparatus, the animal was given intravenously (ear vein) an injection of a solution of sodium phosphate containing P^{32} (0.5 mc. per gram of body weight). Counts were begun immediately over the tumor in each eye and over a portion of the sclera that was free of neoplastic tissue. The measurements of radioactivity were continued for ½ minute in these locations, and the counts were recorded. These results were plotted on graph paper, as in the first phase of this investigation.

The common carotid artery on each side of the trachea was exposed and isolated for a distance of approximately 1 in. Similar exposure of the jugular vein was made only on the side chosen for perfusion. In the animals used for one-half-hour and one-hour studies, the procedure was facilitated if the carotids and jugulars were exposed before or soon after the P^{32} was injected, thereby saving time when perfusion was started. The upper portion of the thorax was then opened, and all branches of the aorta were ligated, except the common carotid artery on the side to be perfused. This ligation was done to prevent any cross circulation either by way of anastomosis or by way of the vertebral arteries. The common carotid artery proximal to the site of perfusion was ligated, and a cannula was inserted in the artery. The perfusion was then begun, with use of a balanced salt solution as the perfusing fluid. This was carried out at 85-90 mm. of mercury for a period of approximately 60 to 70 minutes. Immediately after this arterial perfusion had been started, the jugular vein on the same side was cannulated to secure the venous return. This resulted in a circuit of perfusion that was as closed as could possibly be obtained except by perfusion of the eye directly.

Since the unperfused tumor was to afford some means for standard comparison, this was kept as free as possible from perfusion. Ligation of the common carotid on this side did not seem sufficient, so a fixing substance (barium sulfate-gelatin mixture) was injected into this side of the arterial system, thereby preventing any circulation in this circuit.[42] To prevent any transfer of barium solution via the collaterals to the other side of the perfusion, pressure was temporarily increased during the barium injection so that the perfusion pressure was greater than the pressure on the injected barium stream.

Counts were made every 15 minutes over the intraocular tumors and over the normal sclera in each eye. These results were plotted as before, showing variation in counts for tumors and for normal tissue as a function of time and the ratio of tumor-tissue to normal-tissue counts at various time intervals corresponding to times used for counting measurements in clinical diagnostic tests.

Results.—Results of perfusion studies are illustrated in Figures 17 to 20 and in Table 2, which shows the ratio of the

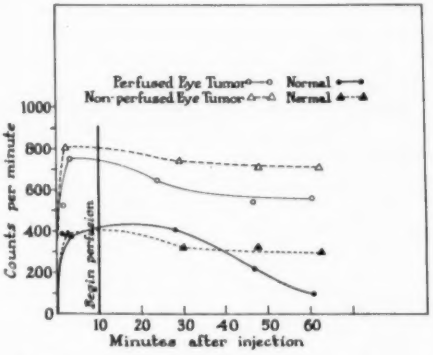

Fig. 17.—Pattern of radiophosphorus uptake in nonperfused normal and in neoplastic tissue (Brown-Pearce carcinoma) and in perfused normal and neoplastic tissue. Perfusion was begun 10 minutes after injection of P^{32}.

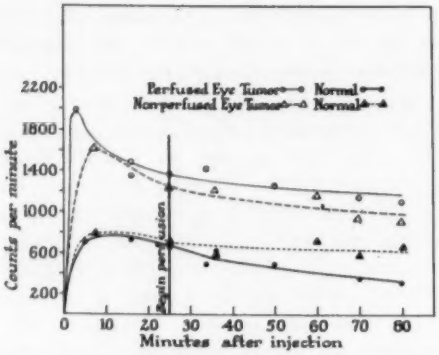

Fig. 18.—Pattern of radiophosphorus uptake in nonperfused normal and neoplastic tissue (Brown-Pearce carcinoma) and in perfused normal and neoplastic tissue. Perfusion was begun 25 minutes after injection of P^{32}.

nonperfused to the perfused eye at the beginning and end of perfusion.

Figure 17 shows the radioactive accumulation in both the perfused and the nonperfused eye and the manner in which these curves were influenced when the perfusion was begun 10 minutes after injection of the radioactive phosphate. At this curve level, P^{32} started to leave the vascular system and continued its exit by way of the buffer solution for the 60-minute time interval. At the time perfusion is started, the uptake curves appear normal, the ratio of tumor count to normal count being 2.0 for the nonperfused eye and 1.85 for the perfused eye (Table 2). The ratio of the

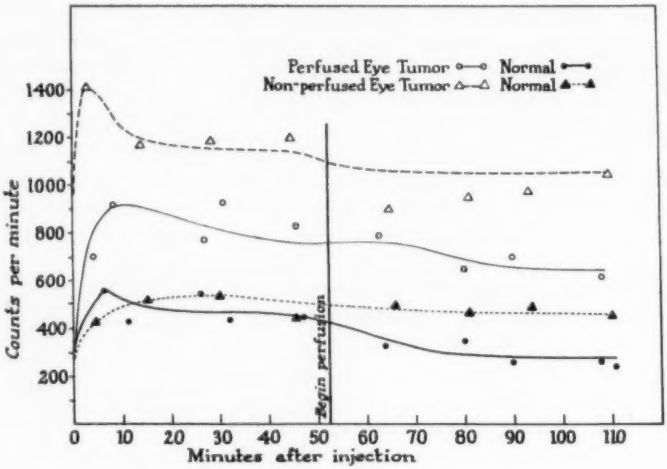

Fig. 19.—Pattern of radiophosphorus uptake in nonperfused normal and neoplastic tissue (Brown-Pearce carcinoma) and in perfused normal and neoplastic tissue. Perfusion was begun 55 minutes after injection of P^{32}.

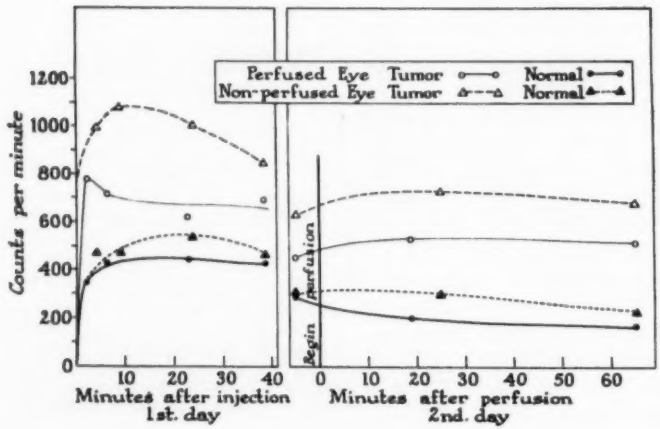

Fig. 20.—Pattern of radiophosphorus uptake in nonperfused normal and neoplastic tissue (Brown-Pearce carcinoma) and in perfused normal and neoplastic tissue. Perfusion was begun 24 hours after injection of P^{32}.

TABLE 2.—*Measurement of Radioactivity, Ratio of Nonperfused Eye to Perfused Eye*

		Time After Injection of P^{32} to Start of Perfusion			
		10 Min.	25 Min.	60 Min.	24 Hr.
Beginning of perfusion	Nonperfused eye	2.0	1.75	2.17	2.1
	Perfused eye	1.85	2.0	2.34	1.59
End of perfusion	Nonperfused eye	2.38	1.54	2.32	2.8
	Perfused eye	5.6	3.6	2.6	3.3

count of the nonperfused eye to that of the perfused eye over the tumor is only 1.05, while the normal is 0.97. These figures show the variation due to "tissue geometry" and are similar, as would be expected. Within 15 minutes after the start of perfusion, the curves have started their decline. The nonperfused eye shows a gradual and normal type of decrease in radioactive output, both over the tumor and over the normal portion of this eye. The perfused eye shows a more rapid drop in radioactivity, reaching a very low level, especially over the normal portion, at the end of the one-hour perfusion. The ratio of tumor count to normal count in the nonperfused eye has changed to 2.38, while in the perfused eye it has greatly increased to 5.6. The tumor ratio of the nonperfused eye to the perfused eye (Table 3) has increased slightly to 1.27, and the similar normal ratio has changed to 3.0. The increased ratio of tumor count to normal count from the beginning to the end of perfusion shows an increase in output of the P^{32} by the perfusion technique, which presumably is due to phosphate not yet metabolized by the tissue. The changed ratio (2.0:2.38) of tumor count to normal count in the nonperfused eye at the beginning and end of perfusion is most likely due to some collateral circulation. The slight increase in ratio of counts over the tumor of the nonperfused eye to that of the perfused eye from 1.05 to 1.27 indicates that phosphorus is bound in the neoplasm and that this radioactive material is removed with difficulty by perfusion. This is in sharp contrast metabolically to the similar ratio for normal tissue, which changes from 0.97 to 3.0, indicating that here the phosphorus is not so rapidly utilized and is still, in contrast, largely free in the circulation.

Curves in Figure 18 show the effects on radioactive accumulation of the perfused and nonperfused eye, over tumor and normal tissue, when perfusion is started 25 minutes after injection of radioactive phosphorus. Until the beginning of perfusion, both tumor and normal-tissue curves show a rapid rise, reaching a peak during the first 5 minutes after injection of the P^{32}, with the decline beginning at the 10-minute point. Accumulation curves in Figure 18 again demonstrate a marked similarity in perfused and nonperfused eyes. Parallel decline in these two curves and the approximately equal differential values at the start and at the end of perfusion are significant. The similar figures in nonperfused and perfused eyes with respect to tumor tissue (Table 3) show that more P^{32} has been bound in the neoplasm than was seen in the 10-minute curve and hence less phosphorus could be recovered by perfusion. The increased ratio in the normal eye tissue at the end of perfusion indicates that here much of the injected phosphorus was still free in the blood stream and could be removed by perfusion.

Figure 19 shows the radioactive accumulation in the perfused and nonperfused eyes, both in the tumor and in normal tissue, when perfusion was begun approximately one hour after the injection of radioactive phosphorus. During the first hour, these output curves follow the expected pattern.

TABLE 3.—*Measurement of Radioactivity, Ratio of Tumor to Normal Tissue*

		Time After Injection of P^{32} to Start of Perfusion			
		10 Min.	25 Min.	60 Min.	24 Hr.
Beginning of perfusion	Tumor	1.05	0.91	1.45	1.38
	Normal	0.97	1.06	1.4	1.3
End of perfusion	Tumor	1.27	0.83	1.16	1.3
	Normal	3.0	1.7	2.06	1.4

As in the previous experiments, the greatest variable is the drop in radioactive accumulation in the perfused normal eye. Shortly after the start of perfusion, this takes a rather definite decline, indicating that there is still considerable "free" or unbound phosphorus in the vascular system. The phosphorus can be removed in part from the normal tissue by perfusion but not to any degree from the tumor tissue.

When perfusion was started 24 hours after the injection of radioactive phosphorus (Fig. 20), calculations of radioactivity again follow the expected pattern for the first 24 hours, dropping somewhat in value but with the anticipated ratios (Tables 2 and 3). There is essentially no change in either the nonperfused or perfused tumor measurement. The only essential difference is again the drop in the normal curve for the perfused eye. This indicates as in the preceding experiments an exit of the radioactive phosphorus in the vascular system by way of the perfusion solution.

3. *Comparison of Uptake of P^{32} and Iodinated Serum Albumin in Tumor and Normal Tissue and Blood.*—The animal was given an intravenous injection of P^{32} and I^{131}, with use of 0.5 mc. of P^{32} per gram of weight and 0.25 mc. of I^{131} (iodinated human serum albumin) per gram of weight. By means of a Geiger tube (Anton No. 222, the end-window of which was covered with lead foil to keep out γ-radiation) and a scintillation tube, simultaneous counts were made over the tumor, with use of a scaler which would record both counts on a line graph.

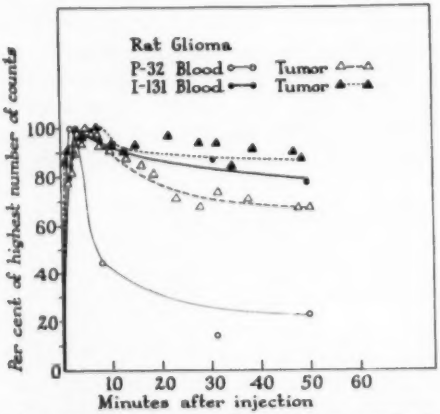

Fig. 21.—Pattern of radiophosphorus uptake in blood and in glioma of the rat. Pattern of iodinated serum albumin (I^{131}) distribution in blood and glioma of rat.

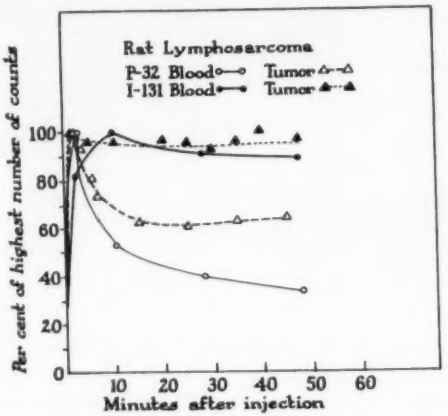

Fig. 22.—Pattern of radiophosphorus uptake in blood and lymphosarcoma of rat. Pattern of iodinated serum albumin (I^{131}) distribution in blood and lymphosarcoma of rat.

Blood samples were taken at various intervals, with use of red blood cell pipettes rinsed into metal crucibles containing about 2 ml. of saline. These samples were dried and counted with a conventional Geiger counter and a scaler. To calculate the amounts of P^{32} and of I^{131} in each sample, two differential counts were made, one with and one without a lead filter.

For studies in vivo, simultaneous measurements were recorded by using both a ¼ in. probe Geiger counter and a ¼ in. probe scintillation counter. Sufficient shielding was placed on the end-window of the Geiger counter to permit passage of the more energetic β-particles of P^{32} while completely absorbing the weaker β-emission of the I^{131} (0.6 mev peak). The γ-ray-counting efficiency of this detector was sufficiently low that the recorded curves could be interpreted as due entirely to the distribution of P^{32} in the tissue.

In a similar manner, the scintillation counter was shielded so as to respond only to γ-emission. Since the conversion of the

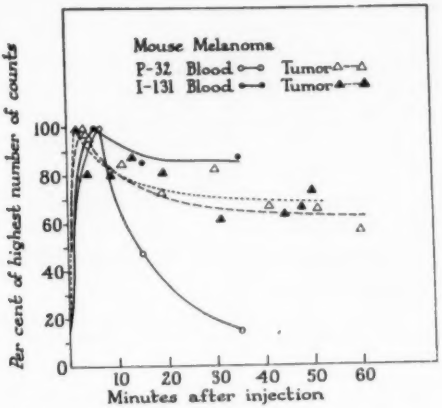

Fig. 23.—Pattern of radiophosphorus uptake in blood and melanoma of mouse. Pattern of iodinated serum albumin (I^{131}) distribution in blood and melanoma of mouse.

β-particle of P^{32} to *Bremsstrahlung* in tissue is slight, this detector may be considered as responding to the I^{131} distribution alone. The separation of the withdrawn blood samples into specific activities of P^{32} and I^{131} was accomplished by performing in vitro assays with separation by differential decay.

Results.—The four composite curves in Figures 21 to 24 exhibit several similarities throughout: 1. Curves representing the concentration of P^{32} in blood reach an initial peak and fall to lower values as a function of time. This indicates that the P^{32} is being lost from the circulating blood and that it must be accumulating in the soft tissues representing the total phosphate space. 2. The curve representing the concentration of the iodinated (I^{131}) serum albumin is much different. The highest concentration of this occurs somewhat later, owing to mixing in the circulation, and from that time on exhibits only a slow loss (10% to 20% for the first hour after injection).

These two known distributions assist greatly in the interpretation of the in vivo curves. Since the iodinated (I^{131}) albumin shows a relative stability in circulating blood, the in vivo I^{131} counting rate is then a direct reflection of the vascularity of the tissue itself. Thus, in the curves representing glioma, lymphosarcoma, and melanoma (Fig. 21-23), the iodinated (I^{131}) albumin distribution in blood parallels the in vivo counting rate to a pronounced degree. These in vivo curves may be interpreted largely as a representation of vascularity alone. The somewhat anomalous results in Brown-Pearce carcinoma (Fig. 24) may be attributed mainly to a difficulty in the in vitro blood sampling.

With the dashed line representing the in vivo P^{32} distribution, these curves contribute the largest amount of information. Since the blood sampling of P^{32} shows a decreasing concentration of P^{32} in the circulating blood and the iodinated (I^{131}) albumin curves show the stability of the vascular measurements, then the in vivo P^{32} measurements may provide indirect information on the accumulation of the phosphate (P^{32}) ion by the tissue in question. These curves, as recorded, show the continual radioactivity present from both intravascular and extravascular regions. Now, since it is observed that the P^{32} distribution in blood is decreasing, the failure of the in vivo P^{32} curves to parallel this decrease must indicate that the difference between these two curves is actually the accumulation of P^{32} in the tumor tissue. This difference is most marked on the mouse

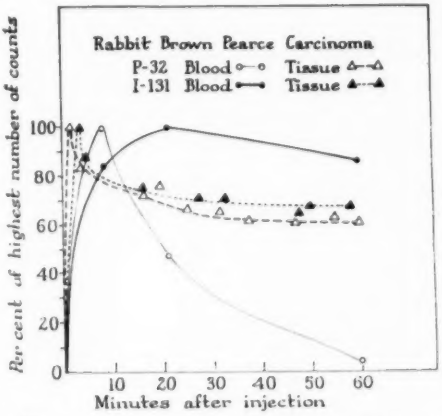
Rabbit Brown Pearce Carcinoma
P-32 Blood Tissue
I-131 Blood Tissue
Per cent of highest number of counts
Minutes after injection

Fig. 24.—Pattern of radiophosphorus uptake in blood and Brown-Pearce carcinoma in rabbit. Pattern of iodinated serum albumin (I^{131}) distribution in blood and Brown-Pearce carcinoma of rabbit.

melanoma and the rat glioma composite curve and indicates heavy accumulation by these tissues. The same effect is noted to less degree in the lymphosarcoma, and a more equivocal result was recorded with the Brown-Pearce carcinoma.

Since these curves have all been normalized to the highest count, it would be possible to subtract the vascular composite of P^{32} from the in vivo curve and have the isolated effect of tissue accumulation alone. Even as represented in actual recordings, however, the differential effect of tissue accumulation is readily seen.

IV. The Biochemical Fate of P^{32} in Normal and Neoplastic Ocular Tissue

In the third phase of our experimental studies an attempt was made to correlate physical-chemical fractionation studies with radioactive counts in normal ocular tissue and in experimental ocular tumors.

Certain interesting physiologic and biochemical differences in proteins, fats, and carbohydrates between normal and malignant tissue furnish the basis for growth studies. By the application of radioactive isotopes as tracers, the fate of the various metabolites and the metabolic pathways taken by these substances can be followed from the injection into the blood stream until the incorporation into the primary cellular structure of the tissue under investigation (Fig. 25).

In our studies we have considered the following groups of nucleic acids: (1) the pentose nucleic acids, or ribonucleic acids

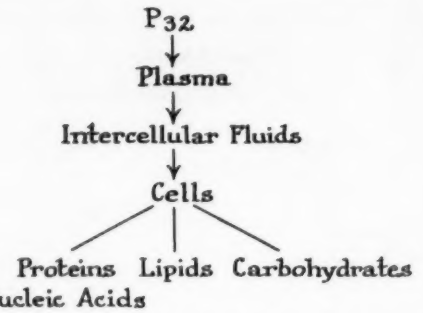

Fig. 25.—Metabolic path of P^{32} from injection into the blood stream to its incorporation into body tissue.

(RNA), and (2) the deoxyribose nucleic acids, or deoxypentose nucleic acids (DNA). In each of these groups there may be a large number of substances, which are situated as follows (Fig. 26): the RNA (or PNA) is present in the cytoplasm and also in the nucleus. Within the nucleus, the nucleoli are particularly rich in RNA and the chromosomes contain only a small amount. In the cytoplasm, RNA is present both in the microsomes and in the mitochondria. The DNA is usually situated in only one part of the cell, namely, in the chromosomes, and its formation occurs predominantly during mitosis.

The results in Parts I and II of this study suggested that the retention of a high level of radioactivity in ocular tumors from two hours to three days after injection of P^{32} was probably due to the high rate of metabolism and cellular growth occurring in the tumor. The following work was therefore carried out to investigate the bio-

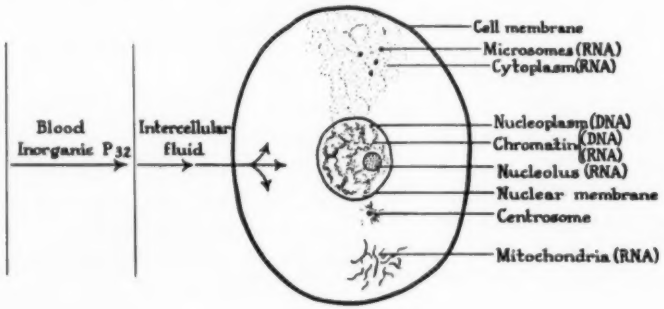

Fig. 26.—Situation of pentose or ribonucleic acids (RNA) and of deoxyribose nucleic acids (DNA) in cellular structures.

chemical fate of P^{32} in experimental ocular tumors.

Method.—The experimental tumors used were the Murphy rat lymphosarcoma, the Brown-Pearce rabbit carcinoma, a rat glioblastoma, and the S-91 mouse melanoma. The techniques used in transplantation and maintenance of these tumors were those described in Part I. Normal ocular tissue consisted of the entire normal eye in the case of the rat and the scleral portion of the eye in the case of the rabbit.

Tumor-bearing animals were given an intravenous injection of P^{32} (0.05μc. per gram of body weight). The tumor and/or normal ocular tissue was removed at various intervals after injection, frozen on solid carbon dioxide (Dry Ice), weighed, and homogenized in cold 10% trichloracetic acid (TCA). The homogenate was then fractionated by a modification of the method of Schmidt and Thannhauser [44,45] into the acid-soluble (AS) phosphorus, phospholipid (PL), ribonucleic acid + phosphoprotein (RNA+PP), and deoxyribonucleic acid (DNA) fractions. "True" inorganic phosphorus and total phosphorus determinations were carried out according to the method described by Umbreit et al.,[46] except that the colorimetric procedure of Gomori [47] was employed. The radioactivity in each fraction was assayed by means of a liquid counter [48] or an end-window Geiger counter.

Results.—Table 4 shows the distribution of P^{32} in the various fractions of four different experimental tumors at various intervals after injection. The results are expressed as percentage of the total P^{32} found in each fraction. During the first two hours after injection, 70%-100% of the total P^{32} was found in the inorganic phosphorus fraction, while 24-96 hours after injection, the P^{32} content of the organic phosphorus fractions (lipids, RNA + phosphoprotein, DNA) accounted for a majority of the total tumor P^{32}. The P^{32} distribution was very similar in the four tumors studied one to three days after

TABLE 4.—*Partition of Radioactivity into Fractions of Intraocular Tumors*

Tumor or Tissue	Time After P^{32}, Hr.	Animals, No.	% Total Radioactivity in					
			Inorganic P *	Total Acid-Sol. *	PL	RNA+PP	DNA	Residue
Rat, glioma	½	2	90.1	97.8	0.2	1.49	0.7	0.45
	1	1	79.7	95	0.6	2.80	0.6	1.0
	24	2	30.0	64	5.5	22.2	7.2	1.3
	72	1	24.7	32.8	26.1	30.1	8.6	2.5
Brown-Pearce carcinoma	5 min.	1	87.8	100	0	0	0	0
	1	3	77.3	84.1	2.12	11.1	1.42	1.26
	2	1	68.0	81.0	3.5	13.3	1.3	1.0
	24	3	24.3	30.9	20.6	35.7	10.4	2.4
	48	2	20.3	28.1	21.1	33.6	15.9	2.6
	72	2	21.4	27.5	21.0	32.3	13.7	5.4
	96	1	33.2	38.9	22.8	20.2	18.1	--
Lymphosarcoma	24	3	17.9	26.1	17.2	40.5	7.32	8.5
	48	2	28.2	36.7	26.3	31.8	3.2	4.0
	72	2	16.7	28.1	14.8	31.9	10.6	14.7
	96	3	16.1	24.2	25.9	29.3	13.9	6.71
Melanoma	½	1	92	95.2	1.2	3.3	0.2	0.1
	1	1	85	93	1.5	4.8	0.5	0.2
	24	1	34.4	49.2	26.1	20.7	3.4	0.5
	72	1	24.8	39	18.5	33.6	8.1	1.8
Rat, normal eye	24	3	46.4	61.3	19.6	10.0	3.9	3.9
	48	2	33.2	51.3	20.7	26.1	0.9	0.9
	72	2	30.6	53.5	14.8	25.1	3.7	3.1
	96	3	22.8	46.0	30.6	15.6	2.6	2.8
Rabbit, normal eye	5 min.	1	72.7	98	0	0	0	2.0
	1	1	84	98	0.6	1.4	0	0
	2	1	74.5	94.5	1.6	4.0	0	0
	25	1	42.4	54.6	11.4	4.1	11.2	18.2

* Total acid-soluble phosphate includes inorganic phosphorus.

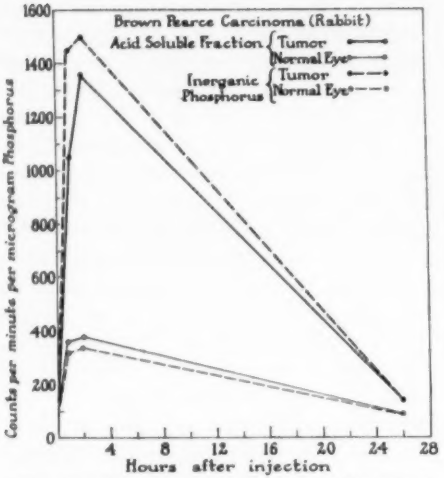

Fig. 27.—Specific activity of acid-soluble and inorganic phosphorus fraction in normal ocular tissue and in the Brown-Pearce carcinoma.

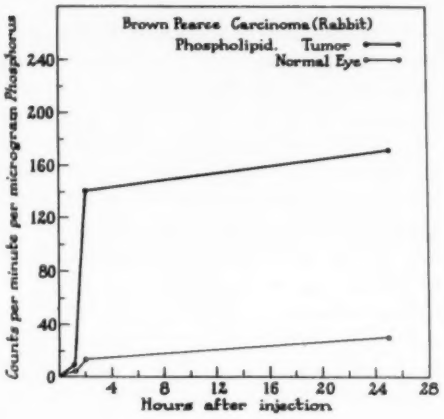

Fig. 28.—Specific activity of the phospholipid fraction in normal ocular tissue and in the Brown-Pearce carcinoma.

injection; the acid-soluble, phospholipid, and RNA fractions each contained about 30% of the P^{32}, and the DNA accounted for the remaining 10%. In contrast, normal ocular tissue contained a smaller proportion of the total P^{32} in the lipid, RNA+phosphoprotein, and DNA fractions and a correspondingly higher amount in the acid-soluble fraction.

Figures 27 to 30 show the specific activity of the various phosphorus fractions of the Brown-Pearce carcinoma and normal ocular tissue of the rabbit at various intervals after injection of P^{32}. The acid-soluble and inorganic phosphorus fractions showed a very rapid rise in specific activity to a level much higher than that of the control, but within 24 hours the specific activity of these fractions had dropped to a level similar to that of the control (Fig. 27). The specific activity of the phospholipid, RNA+phosphoprotein, and DNA

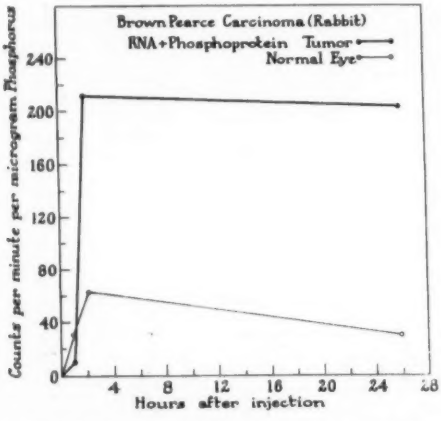

Fig. 29.—Specific activity of the RNA and phosphoprotein fractions in normal ocular tissue and in the Brown-Pearce carcinoma.

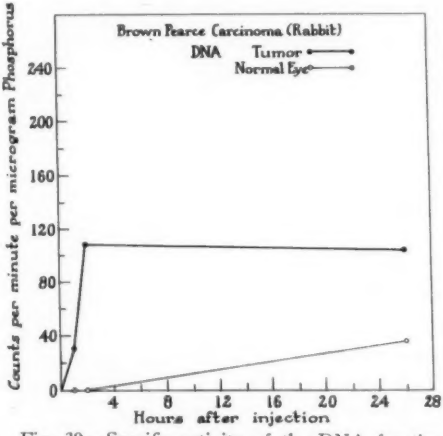

Fig. 30.—Specific activity of the DNA fraction in normal ocular tissue and in the Brown-Pearce carcinoma.

TABLE 5.—*Phosphorus Content of Normal and Neoplastic Ocular Tissue*

Tissue	Animals, No.	P/Gm. Tissue, Mg.					
		Total P *	AS †	Inorganic †	Phospholipid	RNA+PP	DNA
Rat lymphosarcoma	10	2.55	0.78±0.28 ‡	0.53±0.14 ‡	0.34±0.18 ‡	0.68±0.24 ‡	0.22±0.16 ‡
Mouse melanoma	4	3.45	1.18±0.45	0.71±0.14	0.51±0.15	0.60±0.11	0.36±0.04
Mouse glioblastoma	5	1.91	0.57±0.05	0.41±0.26	0.31±0.03	0.37±0.02	0.25±0.04
Rabbit Brown-Pearce carcinoma	9	2.13	0.60±0.25	0.46±0.19	0.30±0.05	0.54±0.23	0.23±0.06
Normal rat eye	10	0.51	0.41±0.23	0.32±0.11	0.16±0.07	0.25±0.08	0.08±0.07
Normal rabbit eye	4	0.32	0.32±0.07	0.28±0.06	0.16±0.05	0.06±0.01	0.08±0.07

* Total P determined by addition.
† AS fraction includes inorganic P.
‡ Standard deviation.

fractions also rapidly rose to values much above the control and remained at the high level throughout the 24-hour interval. The P^{32} appeared to be incorporated into tumor DNA at a much faster rate than into the DNA of the normal ocular tissue.

Table 5 compares the phosphorus composition of the four experimental tumors with normal ocular tissue. All four tumors contained more total phosphorus per gram than did normal ocular tissue of the rat or rabbit. The difference was particularly noticeable with regard to the phospholipid, RNA+phosphoprotein, and DNA fractions.

Comment.—Experiments described in Part I have shown that the radioactivity of experimental ocular tumors was much above that of normal ocular tissue from five minutes to about three days after the injection of P^{32}, whether the measurement was carried out with external probe counters or by in vitro counting of the excised tissue. An inflammatory lesion of the eye (Part II) also was found to show a rapid uptake of P^{32} within 5 minutes after injection, but in contrast to the eye tumors, the radioactivity fell to the control level within 20 minutes after injection. Perfusion studies and comparison of the uptake of P^{32} with that of iodinated (I^{131}) serum albumin led to the conclusion that the early rapid rise in the P^{32} content of neoplastic ocular tissue, as well as of the inflammatory ocular tissue, was primarily due to the high vascularity of these tissues in comparison with normal ocular tissue. The retention of P^{32} by neoplastic ocular tissue over several days, on the other hand, was thought to be caused by the uptake and utilization of P^{32}-phosphate by the rapidly dividing cells of the tumor. The results of the experiments in Part III agree with this conclusion. Thus, during the first two hours, the P^{32} in the tumor was primarily (70%-100%) due to P^{32}-labeled inorganic phosphate, while one to four days after injection, the P^{32} was distributed among the acid-soluble, phospholipid, and RNA+phosphoprotein fractions (30% each) and the DNA fraction (10% of the total radioactivity). The present experiments indicate that the increased level of P^{32} in the neoplastic tissue is due to several factors; high vascularity accounts for the early rise in P^{32} level, thus making available highly radioactive inorganic phosphate for synthesis of organic phosphorus compounds. Since the neoplastic tissue contains more phosphorus per gram than does normal ocular tissue, the tumor would incorporate more P^{32} than the normal tissue even if the synthesis of organic compounds were no faster than the normal. However, as indicated in Tables 1-4, the rate of uptake appears to be somewhat faster in the neoplastic tissue, particularly in the case of DNA, as might be expected because of the rapid rate of cell division.

Comment

In these experimental studies the pathways taken by P^{32} from the time of injection to its distribution in the cellular structure have been studied. By considering the elements of time and amount of radioac-

tive uptake, the influence of various factors, such as tissue geometry and vascularity, upon these readings has been determined. This study points up interesting parallels between experimental and clinical observations in intraocular tumors.

Several points stand out as particularly significant. The validity of the P^{32} test rests upon metabolic change in the involved tissue. Neoplastic tissue metabolizes P^{32} at a more rapid rate than does normal tissue, and the increased readings of radioactive output obtained with the Geiger probe over the neoplasm as compared with the normal are due to physiopathologic changes taking place in that tissue, namely, the more rapid turnover of P^{32} by the cells themselves. These neoplastic cells are growing more rapidly and are more numerous, and hence their utilization of phosphorus is more rapid. The metabolic needs of the two tissues are similar—that is, qualitatively their metabolism is comparable, but the metabolic processes differ quantitatively. This is true with all fractions of tissue phosphorus utilization in the cells themselves and results in significantly different sum totals.

Factors such as tissue geometry and vascularity of tissue are important in proper interpretation of radioactive output, but when these factors are taken into account, results of final readings of neoplastic P^{32} uptake and normal P^{32} uptake are not changed. The tissue geometry explains significant differences in P^{32} readings obtained at various points in the same tumor.

It is likely that the greatest source of error in P^{32} studies lies in incomplete understanding of the physiopathology of cancer tissue and in technical errors related to calculations or faulty instrumentation. The factors here discussed from the experimental standpoint have parallel application in clinical studies of ocular tissues, as has been amply proved by our experience.

Summary

Studies with radioactive phosphorus in experimental tumors were undertaken to determine their application to similar clinical methods. The investigation has been divided into three phases: (1) comparison of uptake of radioactive phosphorus in normal ocular tissue and experimental eye tumors; (2) study of the effect of vascularity on the radioactive uptake in neoplastic, inflammatory, and normal ocular tissue, and (3) correlation of the uptake of radioactive phosphorus with cellular structure and study of the metabolism of phosphorus and its intracellular distribution.

The following tumor strains were used: the Brown-Pearce carcinoma, the S-91 mouse melanoma, the rat glioma, and the Murphy lymphosarcoma. The size of the animal eyes necessitated the use of small Geiger-counter probes, which were especially designed for this study.

The factor of tissue geometry, of great importance in proper interpretation of results, is explained and evaluated.

In all tumors studied, the uptake of radioactive phosphorus was much higher in neoplastic than in normal tissue. The curves of counts per minute, plotted against time of observation, showed an initial rapid rise reaching a maximum in 10 to 12 minutes after injection of NaH_2PO_4, followed by a decline extending over several days. During the initial rise in radioactivity the blood levels of P^{32} were high, and these counts were probably dependent largely on tissue vascularity. After establishment of equilibrium in phosphorus exchange, the decline in curves followed a gradual pattern of decay.

The influence of vascularity upon the uptake of radioactive phosphorus was approached by (1) comparing the radioactive phosphorus uptake by a vascularized inflammatory lesion with that of normal tissue, (2) perfusion studies on eyes containing neoplasms in the anterior chamber, and (3) comparative measurements of uptake of P^{32} and iodinated (I^{131}) serum albumin taken over tumor tissue, normal tissue, and blood. These studies showed that the influence of vascularity lasted only during the first 10 minutes and that after 30 minutes curves of radioactive output then returned

to the usual pattern of decay. The perfusion studies also indicated that vascularity was important only during the early phases of uptake studies. Readings on the uptake of P^{32} and of iodinated (I^{131}) serum albumin showed the P^{32} being lost from the circulating blood and accumulating in the soft tissues, while the iodinated (I^{131}) serum albumin rate reflected the vascularity of the tissue itself. The difference in P^{32} distribution in blood (decreasing) and that shown by in vivo P^{32} curves indicates the accumulation of P^{32} in the tumor tissue.

The metabolic studies consisted of an evaluation of radioactivity in separate fractions of phosphorus at various intervals after injection. During the first two hours, most of the P^{32} can be accounted for as inorganic phosphate, while after 24-96 hours, the P^{32} has been distributed into various organic phosphorus compounds. The increased radioactivity in the neoplastic ocular tissue appears to be due to several factors: the high vascularity making available inorganic phosphorus of high specific activity, the relatively high amount of phosphorus-containing compounds in the tumor, and the rapid rate of synthesis of these compounds.

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A Modified Slit-Lamp Arm

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Modern ophthalmology favors complete examination of the eyes, including routine slit-lamp study with the usual refraction and ophthalmoscopy. This trend has led to more convenient and centralized arrangements of the necessary instruments. Various slit-lamp arms on instrument stands enable the slit lamp to swing easily into position for use without moving the patient after refraction. However, adjustment of the height of the conventional slit-lamp arm is limited. The arms are high enough to require the physician to sit on a high stool and the patient in a high examining chair of the pump or motor-driven type.

It would seem more desirable to seat the patient in a chair of conventional height.

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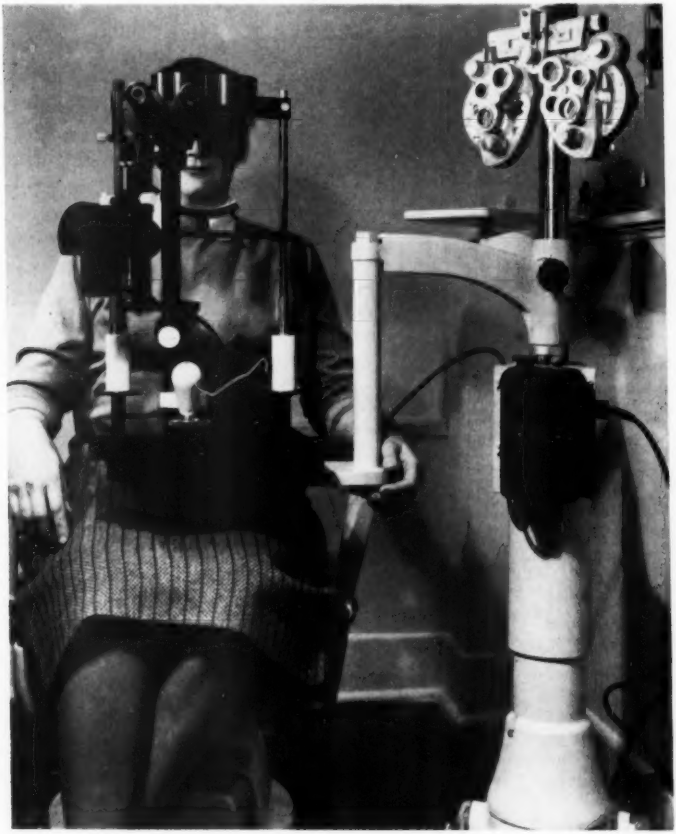
From the Department of Ophthalmology, Ochsner Clinic.

New slit-lamp arm in position.



The refraction and slit-lamp examinations could then be performed at a low enough level to permit the physician to be seated comfortably in an ordinary executive type chair. A newly modified slit-lamp arm that fits on an instrument stand * permits the slit lamp to swing in at a low enough level that both patient and physician can sit at a comfortable conveniently low level. This eliminates the need for the patient to use a cumbersome foot rest, which obstructs easy access to the patient from the front.

The figure shows a new slit-lamp arm † on an instrument stand ‡ with the patient sitting comfortably in a motor chair § without any foot rest; the lowest seat height of the chair is 17 in. from the floor. Other chairs of comparable height can be substituted.‖ With such a slit-lamp arm and one of these chairs, the physician can perform the refraction and slit-lamp examinations while sitting in an ordinary swivel type desk chair. Many ophthalmologists will find this a more comfortable and convenient arrangement.

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* Bausch & Lomb instrument stand.

† The slit-lamp arm is available on order from the Bausch & Lomb Optical Company.

‡ Bausch & Lomb instrument stand.

§ A Ritter Century Model motor chair is shown in the picture.

‖ A Bausch & Lomb motor chair can be easily modified to a low height by removing the adapter ring. A Paidar hydraulic chair is economical and equally advantageous (model with seat height 17 in. from floor, without foot rest, with bar foot pump behind chair and reclining back).

Annual Reviews

The Uveal Tract

J. A. C. WADSWORTH, M.D., New York

Fundamental studies have been made of the uveal tract during the past year. Such studies have contributed valuable information to our better understanding of the role played by the uvea in ocular disease.

Anatomy and Embryology

Wudka and Leopold [1] have continued their study on the choroidal vessels. By direct observation the authors found the motion of the blood in the larger arteries was too rapid to be seen; however, ligation of the homolateral common carotid produced narrowing of the long posterior ciliary arteries. When dyes were injected into the contralateral common carotid, the contrast media appeared in the choroidal vessels under study. Occlusion of the ciliary arteries was followed by compensatory circulatory changes. Occlusion of the vortex veins induced a change in the direction of the blood flow in the affected areas.

In additional studies, these same authors [2] reported the effects on the choroid following treatment for retinal detachment. It was found that scleral-shortening procedures were immediately effective but temporary. A short time after operation a reexpansion of the sclera with tearing of the sutures is the rule. This reexpansion need not be a disadvantage if the retinal hole is closed, because the retina, too, may expand to accompany the sclera. The authors found that the encircling tube or cinch caused definite structural changes which may be detrimental to the health of the eye. Vascular changes in the choroid with atrophy of the iris along with vascularization of the cornea may follow the implantation of the encircling tube. Compression of the ciliary arteries as well as an obstruction of the vortex veins can explain these degenerative changes.

The above findings in experimental animals are confirmed by pathological specimens from humans. The eye usually returns to its normal shape and size, except in those cases where a cinch operation has been performed. These cases remain deformed and constricted by the encircling band.

Berggren and Vrabec [3] have demonstrated a hyaluronidase-soluble substance surrounding the trabeculae. Certain eyes treated show a loss of the hyaluronidase-sensitive barrier, with a resulting swelling of the trabeculae. This important finding may be a cause for wide-angle glaucoma.

Lewallen [4] studied 12 cases of congenital aniridia and found that glaucoma was a common finding; the treatment was not entirely satisfactory, no matter what method was used. Cataracts were common. After due study, extracapsular extraction was recommended. Intracapsular extraction is a dangerous procedure, since most patients are young and rupture of the zonules presents a real problem. Absence of the support from the iris diaphragm adds further danger to an intracapsular extraction.

Persistence of a pupillary membrane is a common finding, but the familial character is not widely known. In one family of 18 cases covering four generations, persistent pupillary membrane was found. Congenital cataract and megalocornea were also associated in the series. It is explained that there is a lag in the disappearance of the membrane during the fourth and fifth

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months of development and the factor causing such a lag is probably genetic (Cassady and Light[5]).

Congenital Defects and Degenerative Changes

Giordulli[6] reported a case of essential atrophy of the iris with normal field and tension. Two years later the patient developed chronic simple glaucoma. The iris atrophy progressed rapidly after the tension rose. From the report of this case, it is difficult to determine whether or not glaucoma had existed prior to the time when the tension rose to a constant high level. Ordinarily, treatment of glaucoma secondary to essential atrophy of the iris is unrewarding, but Daily and Daily[7] reported successful control after iridectomy in the early stage of the disease. Cyclodiathermy is another successful method of treatment to control tension in such cases (Giménez Almenara[8]). An atrophy of the temporal sector of the iris developed in four cases after a Hummelsheim operation for sixth nerve palsy. The operation was followed by a mild iritis and a later development of iris atrophy. Stucchi and Bianchi[9] attributed the iris atrophy to a damage of the blood supply to the iris and suggested a two-stage operation.

Atrophy of the pigment epithelium of the iris was studied in a series of 97 cases by Scheie and Fleischhauer.[10] Transillumination was a valuable diagnostic procedure along with dispersion of pigment deposited in the lens, in the cornea, and in the angle. The iris stroma appeared normal in all cases. Forty-three per cent of the studied cases developed glaucoma secondary to trabecular obstruction from the pigment deposited in the angle. Multiple cysts of the iris produce marked variations in the depth of the anterior chamber. After dilatation the multiple cysts are easily seen, but large cysts are often mistaken for melanoma unless the observer looks for the multiple daughter cysts. Chandler and Braconier[11] reported that glaucoma is frequently associated with these iris cysts. Two cases were reported by Bégue and Jubert-Bélédin[12] where the iris cysts were so large that they caused subluxation of the lens; melanoma was considered in both of these cases. Transillumination may be increased in the area of the cyst and help to establish the true nature of the lesion.

The actual cause of the iris cysts is not clearly known, but Heath[13] suggested that they may be congenital and reported a case of a 5-year-old girl to help substantiate the theory. Small cysts of the pars plana may be seen by ophthalmoscopy, but their presence is thought to have no clinical significance.[14]

Degenerative changes in the choroid following high myopia are thought to be the result of neurovegetative disturbances[15]; however, Raymond and Goodman[16] treated high myopia with corticotropin (ACTH) and lipocaic factors. Lipocaic factors are probably responsible for the improvement that has continued for two years after treatment was stopped.

Choroidal detachment is actually an edematous swelling that often occurs after operation on the anterior segment or injury to the posterior segment with damage to the vitreous body. Scleritis may also cause what appears to be a detachment of the choroid.[17]

Inflammation

Continued study throughout the world is focused on determining the etiology of iridocyclitis. The complete solution is by no means reached, but each year more valuable information is gathered to put us closer to solving this great ophthalmic mystery. More than half of the articles written on the uveal tract pertain to inflammation.

In years past, tuberculosis was considered the chief cause of iridocyclitis, but as our diagnostic acumen has improved, tuberculosis has shown progressive decrease in frequency. Alvaro[18] made a plea for a standardization of tests throughout the world in order that our statistical studies may be more valuable. Burlakova[19] reported a series of cases with iridocyclitis and

found 42.5% to be caused by tuberculosis and 23.2% to be caused by rheumatism. Etiology was not proven in 22.5% and, therefore, was classified as unknown. The methods of proving etiology were not clear. The total percentages failed to reach 100%, but the unaccounted-for 11.8% is probably unimportant anyway. Stanworth and McIntyre [20] analyzed a series of 237 patients with uveitis. In spite of many tests it was difficult to assess the importance of various etiologic factors. However, ankylosing spondylitis and atypical forms of spondylitis was the largest single group of systemic diseases with which uveitis has been associated. Some granulomatous uveitis has been associated with sinus infection. Nongranulomatous uveitis is often associated with strongly positive tuberculin reaction. Krahnstöver [21] reported 12 cases of ankylopoetic spondylarthritis with iritis. Hyperergic and bacterial factors in iridocyclitis showed a correlation between the bacteria in the conjunctival sac and the degree of iridocyclitis. Staphylococci were found in the anterior chamber. Iridocyclitis as a biochemical reaction with toxins was overwhelmingly positive in bacterial iridocyclitis (Kapuscinski [22]). Rudobielski [23] found the leukergy provocative as described by Flock to be tuberculous in origin. The disease was established with clinical certainty. Aqueous punctures in uveitis showed evidence of bacteriophages in about 15% of all cases when evidence of distal foci was present. In two cases of toxoplasmosis, Offret and Saraux [24] found positive serology but no toxoplasmosis in the aqueous. Ogielska [25] induced hyperergic iritis in one group of rabbits and Staphylococcus iritis in the other. The dynamic cytogram was found to be essentially the same in both. The prognosis of the disease may be established by the following index:

$$\frac{\text{Monocytes} + \text{reticuloendothelial cells}}{\text{Leukocytes} + \text{lymphocytes}}$$

In additional work, Ogielski et al.[26] found Kolobolakis' color reaction was negative in a group of animals with hyperergic iritis. This reaction is seen when a solution of dye with silver salts and toxins makes a lasting color that cannot be decolorized with potassium permaganate. In a second group of animals with iritis produced by introducing Staphylococcus pyogenes var. aureus into the anterior chamber, a positive color reaction was obtained in all cases. In humans, nearly all iritis cases showed a positive color test, suggesting a bacterial toxic factor. Streptococci form hemolyzing exotoxin as streptolysin O. Antibiotics formed against the streptolysin can be detected more easily than culture.[27] Bedrossian [28] found that the sedimentation rate is significantly higher in nongranulomatous iritis as compared with granulomatous iritis. Such findings suggest a different etiology in the two diseases. Nongranulomatous iritis is most probably a systemic infection. Hypersensitivity to heavy metals can produce an inflammatory reaction in the eye. Röser [29] reported a central serous chorioretinitis in a patient who was wearing brass dentures for eight years. Rapid recovery resulted after removal of the dentures. Whatever the etiology, there is often an ingrowth of blood vessels into the corneal stroma during the acute phase of anterior uveitis. The vessels tend to recede as the congestion subsides. Visual acuity is not affected by the process. Drozdowska [30] explained the process on the basis of congestion of the ciliary vessels during the active inflammation. The many general tests to determine the nature of uveitis suggest many cases to be of infectious origin. From time to time specific infections are isolated or at least surmised, as in Wilson's report,[31] where among 121 cases of brucellosis there were 7 cases of uveitis.

Continued study on Vogt-Koyanagi-Harada disease exists throughout the world, and there seems to be a general agreement that these conditions are all the same process. Ponte,[32] in his thorough study of two cases, concluded that Vogt-Koyanagi-Harada disease and uveomeningitis are one and the same disease but did not suggest a specific etiology. On the other hand, Yoshida [33] injected subretinal fluid from a case of Harada's disease into the vitreous

of a rabbit and produced a uveitis, but serial passage was not possible. If the subretinal fluid is first injected into a chick embryo, the chorioallantoic fluid becomes capable of producing uveitis even after serial passage through a chick embryo. The author felt that other uveitis viruses might be isolated.

To simplify further the origin of uveitis, Hager [34] concluded that Vogt-Koyanagi-Harada disease and sympathetic ophthalmia is a special form of uveoencephalitis, since all 16 cases of Vogt-Koyanagi-Harada disease reported by Puscariu [35] developed meningeal lymphocytosis. The depigmentation of the choroid, vitiligo, and poliosis were explained on the bases of the influence of the neurotropic agent on the chromatophorotropic element of the pituitary body. Ophthalmic surgery in all uveomeningeal cases is poorly tolerated. Ruiz-Barranco and Garrachón [36] advised that surgery not be done unless absolutely necessary.

A study of Behçet's disease by Sezer [37] revealed the presence of a virus recovered from the subretinal fluid in patients with active inflammation. The virus was also recovered from the blood and urine and grown on chick embryo. Virus recovered from laboratory animals previously infected could be reinjected in animals or grown on chick embryos. Complement fixation was positive 1:126 after infection but less than 1:16 during the active infection or during recurrence. Jadassohn et al.[38] prepared an antigen from a patient with Behçet's disease. Skin tests with this antigen gave a tuberculin-like reaction in infected patients but no reaction in normal controls. Behçet's disease is characterized by relapsing uveitis, recurrent ulceration of the genitalia, skin rash, arthralgia, thrombophlebitis, and neurological lesions. The latter indicates a grave prognosis. A complete picture is not necessary to establish the diagnosis, and it may be years before the complete picture presents itself. Williams [39] followed a case for 10 years before ocular symptoms became apparent. Evans et al.[40] were able to isolate a virus from the eye and brain of a patient who died of the disease. McMenemey and Lawrence [41] reported two fatal cases of Behçet's disease, both of which had neurological complications. Pathologically, the lesions were characterized by small softenings in relation to the small blood vessels of the hypothalamus and brain stem. Pallis and Fudge [42] also found damage in the brain stem, but Wadia and Williams [43] found a widespread involvement of the central nervous system. The authors also suggested that cortisone was of value in the early stage of the disease, but the study is somewhat conflicting. Linton and Saint [44] described a case, in detail, of Behçet's disease where cortisone has suppressed the symptoms after seven years. Eguchi et al.[45] showed that among 13 cases of Behçet's disease there was a state of ariboflavinosis. Thorn's test suggests that there is a relationship between Behçet's disease and adrenocortical function.

Several Greek articles review the literature, and Adamantiadis [46] claims priority in first reporting a complete case of the disease, the recognition of which is now attributed to Behçet. Other Greek articles confirm this by naming the disease Adamantiadis-Behçet's syndrome. The name is the only new thing in the articles.[47,48]

With each succeeding year there is a progressive decrease in the number of published articles concerning tuberculosis of the uveal tract. Once considered a principal cause of uveitis, tuberculosis has taken its relative position as one of many causes for this condition. Of 500 patients with pulmonary tuberculosis reported by Pacynska,[50] 2 cases had choroidal lesions that could be considered tuberculous. These findings correspond to the American, Soviet, and Italian reports, but they are considerably lower than German findings. Ziobrowski [51] reported 28% of phlyctenular keratitis to be due to tuberculosis and 25% of all uveitis to be caused by tuberculosis. Such conclusions were based on clinical findings, course, and several undisclosed laboratory tests. Carli and Huraux [52] found ocular involvement in 12% of adolescent tuberculosis patients. Scattered choroidal lesions did not

indicate a serious prognosis; however, lesions involving the optic disc happen only in serious cases of tuberculosis. Konstas [53] found foci of chorioretinal scars in 13% of inmates of a tuberculosis hospital, but most of the lesions were inactive. These lesions were presumed to be tuberculous. Thirteen per cent incidence of small chorioretinal scars may not be significantly higher than that found in the general population.

Gray [54] emphasized the high degree of tissue reactivity in acute iridocyclitis due to tuberculin. Desensitization in very low doses is indicated. Specific therapy consists of a mixture of streptomycin and dehydrostreptomycin, 1 gm. every second day; paraaminosalicylic acid, 12 gm. daily, and isoniazid, 300 mg. daily in divided doses. The treatment should continue for a minimum of four days. Tuberculin therapy is not without danger, since its administration may provoke an attack of iritis in the sensitized patient. Rachmillewitz et al.[55] confirmed this in a series of patients with polyserositis. Dvorak-Theobald [56] reported a case of acute tuberculous endophthalmitis following a series of desensitization injections for allergy. The patient had had a kidney removed for tuberculous nephritis 15 years earlier. Tubercle bacilli were found in granulomatous areas of the choroid and retina. It is suggested that desensitization therapy may activate tuberculosis. In experimental ocular tuberculosis, ethylthiosulfate inhibited caseation.[57] Bullington and Waksman [58] stated iridocyclitis can be produced in rabbits by the injection of optic nerve tissue from different species plus killed tubercle bacilli and liquid petrolatum (mineral oil). Such a development of uveitis is unique in experimental animals, since no direct trauma to the eye was necessary.

Sympathetic Ophthalmia

Steroid therapy has apparently been a great addition to the effective treatment of sympathetic ophthalmia. Many authors report successful treatment with the use of steroids. Minnes [59] discussed the treatment of six cases of sympathetic ophthalmia, and he believes that early vigorous intravenous treatment with corticotropin is the method of choice. Svěrák [60] also used corticotropin with favorable results, and, on the strength of his success, the author suggested a more conservative attitude toward excision of the sympathizing eye. It may be necessary to remove the sympathizing eye before steroids are effective. Cortés de los Reyes [61] reported a boy of 8 years who developed sympathetic ophthalmia after a contusion injury. Finally the injured eye was removed, and histologic diagnosis was confirmed. Corticotropin was given intravenously, and the remaining eye became quiet. It is possible for local cortisone to produce an improvement in the signs of sympathetic ophthalmia, as suggested by Pacurariu and Cîmpeanu-Codreanu [62]; however, the two cases in question developed sympathetic signs after enucleation of the injured eye. Those cases that developed sympathetic ophthalmia before enucleation failed to respond to local treatment. Such results lead one to question the diagnosis of sympathetic ophthalmia in the first place. The value of steroid therapy as a prophylaxis is questionable and may not be in the best interest of the patient. McLean [63] felt that steroid therapy should be withheld unless specific indication is seen for its use. In some instances, Irgapyrin has been credited for clearing a sympathetic ophthalmia when all other treatment failed. It is interesting to note, however, that Irgapyrin was not started until after the offending eye had been enucleated.[64] Hrabi [65] reported a case of intractable uveitis that occurred 20 years after perforating injury to the fellow eye. No pathologic report was given, so the true nature of the uveitis was not known. Electron-microscopic examination of eyes with sympathetic ophthalmia that were enucleated showed virus-like corpuscles 40-75 mμ in diameter within the epithelial cells. Such findings suggest a virus to be the causative agent for sympathetic ophthalmia.[66]

Toxoplasmosis

In a comprehensive review of ocular toxoplasmosis by Frenkel and Jacobs,[67] there is

an organized account of the disease, with a review of the literature. Conclusive proof is given of ocular toxoplasmosis, and the authors suggest the frequency of the disease. The large reservoir of undiagnosed cases should be studied in order to isolate other groups of specific infections. Chorioretinitis may be the only manifestation of the disease in congenital toxoplasmosis. In fact, the purely ocular form is the commonest variety of the disease. Toxoplasmosis is one of the most widespread diseases of mankind, and, by this premise, the specificity of serologic methods is thereby enhanced. Fair [68] recommended that serologic testing of all mothers be done in the investigation of all cases of chorioretinitis where toxoplasmosis is suspected. Saugstad [69] reported a case of a 9-year-old girl who developed choroiditis with a positive dye test of 1:600. After a 10-day treatment with pyrimethamine (Daraprim) and sulfadiazine, the dye test was positive only 1:48 and the choroiditis healed. The mother and siblings were negative; therefore, this case was classified as an acquired one. Pillat and Thalhammer [70] reported a case of granulomatous uveitis in a boy of 11 years. The skin test and complement-fixation test supported the diagnosis of acquired toxoplasmosis. Inoculation of ocular tissue into mice gave a strong reaction. The authors claim this to be the first documented case of iridocyclitis due to acquired toxoplasmosis. In a series of 261 cases of uveitis, the incidence of positive Toxoplasma dye test was higher than expected in the normal population. Pyrimethamine therapy showed better results in these cases with positive dye test than those with negative test. It was concluded by Perkins and Beverley [71] that such cases were due to toxoplasmosis. Kessel [72] used experimental animals to prove that the dye test is specific to Toxoplasma. Helminths and protozoa may often cause uveitis, but toxoplasmosis is the commonest protozoan infection.[73]

Miscellaneous Infections

Specific infections of the uveal tract have been reported in articles from all over the world. Some conditions are rare but at least help to solve the complicated problem of diagnosing uveal inflammation. Coccidioidomycosis causes a granulomatous uveitis, and Brown et al.[74] reported a case in which histological study revealed spherules surrounded by lymphocytes and plasma cells. The organisms were typical of coccidioidomycites. Mycosis fungoides may also produce a granulomatous uveitis, as reported by Gärtner.[75] Leptospiral infection has a world-wide distribution, with a higher incidence in Europe than in the United States. The systemic disease caused by a spirachetal blood-borne infection is capable of lodging in any organ of the body, consequently the multiplicity of symptoms. Jaundice commonly occurs when there is gross involvement of the liver, but jaundice may not be present. Rowen [76] reports that uveitis often accompanies infection. The onset is sudden and severe, but usually recovery is without loss of vision. Organisms may be recovered from either the conjunctiva or the aqueous. The treatment is supportive.

Leprosy often causes uveitis, most frequently involving the anterior part of the eye.[77] Bouzas [78] reported that the use of hydrocortisone injected subconjunctivally results in a dramatic clearing of the inflammation in a matter of days.

Herpes virus has been recovered from adjacent skin lesions in a case with iritis. The uveal inflammation appeared prior to the skin lesions. After transfer of the virus to experimental animals, it was concluded by Hewson [79] to be herpes simplex.

Certain systemic diseases produce an inflammatory reaction in the uveal tissue. Fifty per cent of cases with sarcoidosis have ocular manifestations. Sarcoidosis should be suspected in all cases of granulomatous iritis with negative tuberculin reaction. According to Green and Kennedy,[80] isoniazid and cortisone offer the best therapy at present. Wegner and Würm,[81] in a monograph on sarcoidosis, favored the theory that its etiology was a benign tuberculosis with high immunity and low allergic activity. Fisch-

er[82] studied 500 patients with diabetic retinopathy, and only 4 were found to have choroidal changes. The author believed there were similar changes in the choroidal vessels as found in the retinal vessels. Diabetic iritis is thought to be caused by the presence of neutral fats and cholesterol in the anterior chamber. Insulin leads to a rapid absorption of the lipoidosis and clearing of the iritis.

Woods and Stone[83] selected 58 patients from a group of 900 patients with uveitis. A complete survey as advocated by Woods was carried out. In this study the patients with various forms of uveitis revealed no undue incidence of clinical or subclinical collagen disease. In addition, a review of patients seen with lupus erythematosus, periarteritis nodosa, or scleroderma failed to reveal any incidence of uveitis associated with these diseases. There is no apparent relationship in the electrophoretic pattern of the serum globulins, except in patients with systemic granulomatous disease and eye involvement. The decrease in γ-globulin may be related to a depression of antibody formation or of systemic immunity.

Iritis due to lens material in the anterior chamber should be treated by the removal of the lens matter in spite of the inflammation, according to Witmer.[84]

In the treatment of iritis, Morosov[85] suggested small doses of x-ray. Doses of 10-30 r at one- to three-day intervals gave good results. No more than 250 r was given in any case. Scassellati-Sforzolini[86] found that the injection of 5% buffered solution of sodium salicylate blocks the onset of anaphylactic uveitis more effectively than cortisone. Intravenous administration of typhoid vaccine undoubtedly stimulates the pituitary-adenocortical system and causes an increase of the urinary elimination of neutral 17-ketosteroids, which last for about 48 hours. Krudysz and Kleczenski[87] found this reaction similar to the observations made in patients with rheumatic fever, rheumatoid arthritis, and subacute bacterial endocarditis treated with hypoglycemic insulin shock.

Neoplasms

Far more is written about melanomas than any other neoplasm involving the uveal tract. In spite of its comparitive rarity, much work has been reported on tumors of the iris. Rones and Zimmerman[88] studied a large series of iris tumors. Primary tumors are relatively rare and account for less than 1 out of every 15 uveal tumors. About two-thirds of the iris tumors are discrete and can be removed by iridectomy. Such tumors resemble nevi, leiomyomata, or spindle type melanoma. Some resemble epithelioid type melanomas. It was found in their study that a completely excised tumor eliminates the justification for enucleation; however, if study reveals tumor remaining in the iris or ciliary body, enucleation should be advised. Conservative management is recommended because life expectancy is usually not affected. Cleasby,[89] in his study of iris melanomas, recommended conservative treatment, too. Bakbardin[90] reported the diagnosis of a case of iris melanoma by means of electrocautery and diathermy. A portion of the ciliary body was excised with the tumor, and no metastasis or recurrence has been noted for two and one-half years. From the report, the excision seems radical and extensive and a two and one-half year follow-up is not conclusive in the study of melanomas. In an analysis of 43 cases of primary tumors of the iris, Duke and Dunn[91] found 28 to be melanomas, and one patient died of metastasis. Of the remainder of cases, 13 were benign melanomas and 1 was a leiomyoma. In the light of other studies, the rarity of leiomyomas is surprising; however, the histological differentiation between melanomas and leiomyomas may be very difficult. The diffuse melanoma of the iris is an extremely malignant growth and should be suspected if, on examination, one iris has acquired more pigmentation than the other. Glaucoma in the affected eye helps to confirm the diagnosis. Gonioscopy, radioactive-phosphorus-uptake studies, and biopsy may fail to yield conclusive evidence of the diagnosis.[92]

Cross [93] reported a case of iris tumor that resembled a melanoma clinically but histologically suggested a leiomyoma. The benign course of some iris melanomas suggests leiomyoma, and many cases should be reviewed with special stains to determine the true character of the tumor. Brown and Tüzmen [94] emphasized the difficulty in recognizing leiomyoma. Nevus of the iris is relatively common, but a nonpigmented nevus in children is rare. Weinstein,[95] however, reports an actively growing nevus of the iris in a child of 10 years.

Melanomas of the uvea act differently, and the follow-up is unpredictable. Anderson and O'Neill [96] reported a study of 14 cases of intraocular melanoma. In some, enucleation was refused and metastasis was delayed or absent for many years. Some patients developed recurrence locally or metastasis years after enucleation. Such variable action of this tumor suggests additional factors that influence the future growth of metastasis. In pigmented melanomas a dark shadow of the edge of the tumor is clearly visualized by retroillumination. Such a simple test is a valuable diagnostic measure. By such examination, Reese [97] correctly diagnosed 85% of 214 cases suspected of melanomas of the choroid. Mayer and Beitman [98] reported a case of melanoma of the choroid in which the patient survived 26 years after enucleation and finally died with generalized metastasis. Other such cases have been reported. I have studied a case that developed local recurrence over 30 years after enucleation of an eye that showed evidence of extraocular extension. The patient died one year after the local recurrence appeared. Jensen [99] reported a patient with choroidal metastasis from a melanoma of the skin. Numerous other metastases were also present. Hopkins and Carriker [100] studied a series of cases with melanoma of the ciliary body in an effort to correlate the difference from choroidal melanoma. No statistical data could be obtained from the small series. Discussion of the signs and symptoms brought to light the fact that coexisting cysts in the tumor may cause transillumination to be negative. Gonioscopy shows increased pigment in the angle and is a helpful diagnostic sign. Blind glaucomatous eyes may harbor an unrecognized melanoma of the choroid.[101] Wolter and James [102] reported a case of diktyoma of the ciliary body in a patient of 39 years. The tumor occurred in a blind phthisical eye. Aspiration of fluid from what appears to be a choroidal detachment may reveal a melanoma. Brückner and Ludin [103] emphasized the importance of studying ocular fluids for the presence of tumor. Cellular deposits on the posterior surface of a detached retina in the presence of a choroidal melanoma are the result of necrotic pigment epithelial cells as well as tumor cells in the subretinal space. Hagedoorn [104] recommended this as a helpful diagnostic sign, but in acute choroiditis with retinal detachment the deposits may also be present. However, Hagedoorn and Salim [105] described the proliferation of pigment distal to a choroidal melanoma. Such pigment deposits are due to the stimulating effects of the toxic subretinal fluid on the pigment epithelium. Scassellati-Sforzolini [106] treated a series of choroidal melanomas with diathermy and promptly enucleated the eyes for study. Histologically, diathermy did not consistently destroy the tumor. The author recommended that diathermy be limited to small flat tumors. Treatment of individual intraocular tumors must be varied to suit the case under study. Dunphy [107] suggested local excision of iris tumors if the neoplasm does not extend to the ciliary body and is localized. In retinoblastoma, it is necessary to examine the fellow eye at frequent intervals for at least three years. Nodular iridocyclitis due to retinoblastoma may be a diagnostic problem. Chams [108] reported such a case that developed hypopyon and glaucoma. Histological study revealed diffuse retinoblastoma. Küchle et al.[109] stated that when retinoblastoma involves the ciliary body iridocyclitis may develop and obscure the true nature of the disease. Anterior chamber puncture may be helpful. Bone-free x-ray of the eye is a well-established diagnostic aid. Electroretinogram studies on

intraocular tumors were of no value in the differential diagnosis.[110]

Metastatic carcinoma to the choroid most commonly originates from the breast. Dickson[111] stated that 70% of all metastatic tumors to the choroid arise from the breast. X-ray therapy often gives visual improvement, which may persist for the life of the patient. The average life expectancy of a patient with choroidal metastasis is 10 months. Hotz[112] reported a case with generalized metastasis of a breast carcinoma who was treated by oophorectomy. Vision improved temporarily, but new ocular metastases developed. Blondet et al.,[113] however, reported a similar case, except that adrenalectomy plus oophorectomy was done. Choroidal metastases disappeared, but the length of follow-up is not known.

Occasionally, metastasis may involve the iris. Cury[114] reported a rare metastasis to the iris from a squamous-cell carcinoma of the lower lip. Histologically, the intraocular growth resembled epithelization of the anterior chamber.

Usually angioma of the choroid has no diagnostic sign; however, Böke[115] reported three cases in which associated facial angiomata suggested the true nature of the choroidal mass. Boulanger[116] felt that angiomata of the choroid are often associated with infantile glaucoma. Progressive hyperopia, to as much as 10 D., has been seen in a bilateral case of choroidal angioma.

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Correspondence

APPLICATION OF POISEUILLE'S LAW TO AQUEOUS OUTFLOW

With regard to this article (McEwen, W. K.: Application of Poiseuille's Law to Aqueous Outflow, A. M. A. Arch. Ophth. 60:290-294 [Aug.] 1958), cursory consideration may lead to the conclusion that the so-called "theory of the aqueous flow through the trabecular meshwork" or "Friedenwald's theory" represents a masterpiece *sui generis*, whereas in reality it is just applied hydrodynamics. Its keystone and fundamental term, "facility of outflow," does not represent a new characteristic but is identical with the classical "coefficient of permeability" or "transmission constant"—called in the pertinent German literature "Durchlässigkeit" or "Hydrogeologische Konstante" to point to the field of practical application, namely, the flow of fluids (water and oil) through porous material (sands, sandstones, and porous rocks), including the flow of water through filter beds for municipal water systems. The unit of the "permeability" or the "facility of outflow" is named 1 Darcy, after the engineer H. Darcy, the author of the famous tractate,[8] *Les Fontaines publiques de la ville de Dijon*, 1856.

Darcy's classical experiments have led to the simple result that the rate of flow of water $\frac{v}{t}$ (v=velocity in centimeters per second, t=time in seconds) through a filter bed (trabecular meshwork) is directly proportional to the area, A, of the sand (trabecular meshwork) and the pressure difference, p, between the fluid heads at the inlet and outlet faces of the bed (between the intraocular pressure and the episcleral venous pressure) and inversely proportional to the thickness, L, of the filter (trabecular meshwork), or expressed analytically:

$$(1)\ \frac{v}{t}=\text{const.}\frac{1}{\eta}\cdot A\cdot\frac{\Delta p}{L}\quad\text{or}$$

$$(2)\ v=\text{const.}\frac{d\,p}{d\,x}\quad\text{for } A=1\text{ cm.}^2\text{ and } t=1\text{ second.}$$

Equation 2 represents Darcy's law in its simplest form and states that the velocity of a fluid flowing through a porous medium (trabecular meshwork) is directly proportional to the pressure gradient $\frac{d\,p}{d\,x}$ acting on the fluid (aqueous). Written in this form Darcy's law is self-evident.

The "const." of Equation 2 is of complex nature and represents the "effective permeability," is identical with the ophthalmological term "facility of outflow," and measures the volume of fluid filtering through a unit cross section in unit time under the action of a unit pressure gradient. Its unit is known as 1 Darcy.

In its explicit form the effective permeability can be written

$$\text{const.}=k\cdot\frac{\zeta g}{\eta}\left(\frac{\text{cm.}^3/\text{second}}{\text{cm.}^3\cdot(1{,}033,\ 2\ \text{gm/cm.}^3)/\text{cm.}}\right)$$

That means it equals the "permeability k stricte sensu" times the factor

$$\frac{\zeta g}{\eta}$$

where ζ=density of the fluid (gm·cm.$^{-3}$)
g=acceleration of gravity (cm·sec.$^{-2}$)
η=viscosity (poise)

In the literature one can find the "const." of Equation 1 as well as the "const." of Equation 2 denoted as so-called "permeability." That means the cursory term "permeability" can be defined [1-7] with or without the implicit factor $\frac{1}{h}$.

To consider the viscosity, η, of the aqueous humor as a constant (naturally except for temperature) is equivalent with the statement that the minimal alteration of η caused by the additional energy dissipation through the viscous action of minute amounts of mucopolysaccharide-like substances (which vary in disease) can be neglected.

Furthermore, we do not pay attention to the interesting "surface-tension forces," [4,5,9] which are also closely related to variations of η.

Another even more complicating factor which renders the introduction of the permeability as a basic value into the mechanism of outflow of aqueous through the trabecular meshwork (including Schlemm's canal) hypothetical, is the embarrassing interference of local pressure changes in the filter, which modify size and shape of the elastic channels during measurements on the living eye, e. g., during tonographic studies, when a plunger impinges the cornea.

Before taking in consideration the application of any one of the hydrodynamic laws—Darcy's law, Poiseuille's formula, or Bernoulli's law—it might appear worthwhile to emphasize that each law or formula has very specified limitations. The discourse or error calculus centering around these limitations is by far more elucidative than rushing into cursory evaluations of numerical data, which are at present time more or less overloaded with hypothetical propositions. We have good reason to pay attention to these preambles, because from the aforementioned paper of Dr. McEwen we have to take it as granted that the quantitative details of the trabecular meshwork are so well founded as to enable us to do a further step and to apply a specification of Darcy's law, namely, Poiseuille's formula, to the mechanism of the aqueous outflow. From the analytical standpoint this would have the practical meaning that the requirement of lamellar flow as a *conditio sine qua non* for the application of Poiseuille's formula would no longer represent an embarrassment for experiment and theory.

To guarantee "Poiseuille's flow," that is, a motion of fluid in lamellar streamlines, the microscopic tubes have to fulfill the following requirements:

1. They have to be straight and not tortuous like the channels of the trabecular meshwork;
2. They must be smooth and should not have pits and holes like the lamellae of the trabecular meshwork;
3. They ought to have a circular circumference and should not vary in size and shape like the lacunar interspaces, and
4. They have to be stable, that is, able to resist distortion like the glasspipes used by Poiseuille, and should not be flexible like the elastic walls of the lamellae.

In "Poiseuille's flow" the velocity is essentially parabolic across the section of these ideal tubes falling from a maximum at the center to zero at the walls, and the total flux is proportional to the square of the diameter of a channel.

In "Darcy's flow" of fluid through a porous medium the velocity is uniform over the cross section of the filter, and the total flux is proportional to the first power of its area.

These discrepancies between theory and reality will lead us to contemplate on the limitations inherent in the application of these "laws." In order to give the following discourse a more practical aspect, I would like to point out the difference between the idealization Dr. McEwen is introducing and the real properties of the outlet channels. Dr. McEwen considers the mechanism of outflow of aqueous through the trabecular meshwork as a soluble problem of the classical hydrodynamics where there are well-defined tubes with inflexible and impermeable boundaries, whereas in reality we have to deal with elastic flow channels composed of an intricately meshed system of fine passages characterized by bizarre multiple lateral interconnections.[10]

While Dr. McEwen is handling the outlet channels analytically as equivalent to a series of straight, inflexible, and smooth tubes of a length of 20μ and a diameter of a few microns, it must be remembered that the trabecular meshwork consists of a labyrinth of irregular tortuous channels resulting in a picture which is closer to a sand-filter than to the visualization presented by Dr. McEwen.

The trabecular meshwork may appear to provide serious limitations to the application of the results of classical hydrodynamics, since large parts of the trabecular system may derive their aqueous-carrying capacity largely from discontinuities and irregularities or "fractures" of the trabeculae. It may turn out that the dynamics of those "fractures," which represent disconnected elastic lamellae, is more elucidative for the mechanism of the outflow of the aqueous than the ideal tubes of Dr. McEwen. These fractured lamellae fulfill all requirements which

allow them to function like valves, that is, to collapse with increasing flow governed by the law of Bernoulli. This is not the place to introduce the application of Bernoulli's law to the outflow of the aqueous through the trabecular meshwork. It may suffice to stimulate the attention to a little experiment which simply consists in holding two sheets of paper not too closely together and blowing air into the interspace. The sheets do not separate but become adherent despite the flow of air through the microscopic interspace.

In the case of the eye it is hard to believe that the required hypothetical streamline character of the flow of the aqueous through a labyrinth of tortuous channels can be maintained over a distance great enough to justify the application of Poiseuille's formula, whereas the aforementioned fractured lamellae might be elastic, smooth, straight, and close enough to each other to enable them to function as microvalves obeying Bernoulli's law.

The limitations of Poiseuille's formula, which are based upon the nature of the filter (diameter of the outlet channels) as well as upon the characteristics of the fluid (velocity and viscosity) find a pregnant expression in the analytical term

$$\frac{d \cdot v \cdot \zeta}{\eta} \quad (d = \text{diameter};\ v = \text{velocity};\ \zeta = \text{density};\ \eta = \text{viscosity})$$

known as "Reynold's number." The Reynold's number represents a criterion as far as the nature of flow changes its character from a laminar to a turbulent motion if Reynold's number increases so as to exceed a critical value. In the experiment this critical limit has been found lower for porous material than for tubes[6-8] which fulfill the requirements of Poiseuille's formula. How much Reynold's number in the case of the trabecular system is lowered below the critical level which is consistent with tubes of macroscopic dimensions, we do not know because of lack of experiments with porous media similar to the trabecular meshwork. The sharp variation and irregularity of the cross section of the channels represent a condition which is conducive to the early establishment of turbulent flow.

Summary

Any analytical work attempted in the field of the mechanism of the outflow of the aqueous through the trabecular meshwork is based upon certain necessary assumptions and limitations regarding the nature of the porous structures. A thorough consideration will lead to the conclusion that the limitations are so definite and the introduced assumptions—when compared with the classical laboratory experiments of the physicists—so unrealistic as to prevent the application of a "law," which, like Poiseuille's formula, is founded on the exact knowledge of so many details characterizing the structures of the trabecular meshwork as to render the attainment of quantitative results from any mathematical analysis problematic if not misleading.

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To the Editor:—Thank you for the opportunity of replying to the comments of Dr. Lohle regarding my recent manuscript.

The general tenor of Dr. Lohle's comments is that Poiseuille's law when applied to flow through the trabeculae yields results which are "problematic if not misleading."

That the results are problematic in the sense that there is still a problem, I quite agree. There is always uncertainty in applying any law developed in a physical milieu to a biological system. I felt that the readers of this journal were sufficiently acquainted with the uncertainties and difficulties of biological research that they needed no more than the note of caution given in my paper. If I have underestimated their familiarity with medical sciences, Dr. Lohle's comments should further warn them. The purpose of the paper was not to arrive at any precise quantitative values but to use recently acquired data to carry along the work of previous distinguished investigators who have used Poiseuille's law, in the hopes of arriving at useful and original concepts of the flow through the trabeculae. If the concepts are useful in correlating previous data and in suggesting new experiments, then the work is worthwhile.

I am pleased that Dr. Lohle brought forth no real evidence of any specific errors or misleading statements, but I regret that he did not suggest any formulation by which the factors regarding the flow through the trabeculae could be computed with more certainty. In view of this lack of specific criticism, I can only assume that he feels I was the fool who rushed in where angels feared to tread. I found the journey interesting.

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News and Comment

ANNOUNCEMENTS

Postgraduate Course at University of Michigan.— The University of Michigan Medical School will hold a postgraduate conference in ophthalmology at Ann Arbor, Mich., April 20-22, 1959. Programs will be mailed as soon as they are completed. For further information, write to Dr. John M. Sheldon, Director, Department of Postgraduate Medicine, Room 1610, University Hospital, Ann Arbor, Mich.

Program in Ophthalmic Surgery, St. Louis.—Ophthalmologists from the western and southern states are invited to an extensive program in ophthalmic surgery to be presented at the Kiel Auditorium and local hospitals in St. Louis, March 11 and 12, 1959, as a part of the program of the four-day Sectional Meeting of the American College of Surgeons. This program is being organized by local ophthalmic surgeons under the leadership of Robert D. Mattis.

Sessions at the Kiel Auditorium will include a symposium on Ophthalmic Surgery of Trauma, with Erwin E. Grossman, Milwaukee; Merrill J. Reeh, Portland, Ore.; Vincent L. Jones, St. Louis; Phillip Ellis, Little Rock, Ark., and Ichiro D. Okamura, Boston, as participants.

Another symposium on Ophthalmic Surgical Infections will be presented by Henry F. Allen, Boston; William B. Clark, New Orleans; M. Hayward Post, St. Louis; W. Howard Morrison, Omaha; Roy E. Mason, St. Louis, and Albert N. Lemoine Jr., Kansas City, Mo.

A detailed program in ophthalmic surgery to be presented in St. Louis hospitals will be distributed at the registration desk during the meeting.

The St. Louis Ophthalmological Society has scheduled a special dinner for visting ophthalmologists on Tuesday, March 12, in the Tiara Room of the Park Plaza Hotel. Dinner tickets will be available at the registration desk.

Detailed information about the program and hotel reservations may be obtained by writing to the Department of Organization and Assembly, American College of Surgeons, 40 E. Erie St., Chicago 11.

Program in Ophthalmic Surgery, Montreal.—Ophthalmologists from Canada and the eastern United States are cordially invited to a Sectional Meeting of the American College of Surgeons to be held in Montreal, April 6-9, 1959.

Extensive programs in ophthalmic surgery are to be presented at the Queen Elizabeth Hotel and at local hospitals, April 6-8. These programs are being arranged by a committee of ophthalmic surgeons in Montreal, headed by John C. Locke. Visting speakers will include A. G. DeVoe, I. S. Jones, A. F. DeRoetth, J. H. Dunnington, and R. T. Paton, all of New York; C. C. Johnson, R. Trotter, and P. A. Chandler, all of Boston, and A. D. Ruedemann Sr., A. J. Elliot, and H. L. Ormsby, all of Toronto.

Hotel sessions will also include the following papers by Montreal participants: "Cataract Extraction with the Use of Alpha-Chymo-Tripsin," by Roland Cloutier; "A Clinical Observation in Central Serous Retinopathy," by Harry Magder; "Microphthalmos with Orbital Cyst: Case Report," by Michel Mathieu.

Programs will also be presented at the Royal Victoria, Montreal General, and Montreal Children's Hospitals. These sessions will include a round-table conference on Detachment of the Retina, with Samuel T. Adams, Robert J. Brockhurst, P. Robb McDonald, L. Harrell Pierce, and Donald M. Shafer as participants.

The following papers will also be presented by Montreal participants: "Results of Treatment of Dacryocystitis in Children," by R. Alan Bourne;

"The Collection, Storage, and Selection of Human Vitreous for Use in Retinal Detachment Surgery," by G. Keith Edwards;

"Results of Surgical Treatment of Traumatic Cataract in Children," by Alfred J. McKinna;

"Ocular Aspects of Head Injury," by Sean B. Murphy;

"Office Management of the Patient with a Reading Difficulty," by John V. V. Nicholls;

"Radiation Therapy of Diseases of the Cornea: Ophthalmological Aspects," by William Turnbull;

"Radiological Aspects," by Jean Bouchard.

Detailed information about the program and hotel reservation forms may be obtained by writing to the Department of Organization and Assembly, American College of Surgeons, 40 E. Erie St., Chicago 11.

Meeting of Alumni Association of New York Eye and Ear Infirmary.—The Annual Spring Meeting of the Alumni Association of the New York Eye and Ear Infirmary will take place April 20-23, 1959, at the Infirmary.

Symposia will be offered on headache, complications of ocular surgery at the operating table and their management, management of postoperative complications (24 hours to 2 weeks), practical application of new therapeutic measures in ophthalmology, and ophthalmic plastic surgery. There will also be a closed-circuit television demonstration of surgical operations.

In addition, courses will be offered in the following subjects: recent advances in biomicroscopy, contact-lens fitting, external eye diseases, indirect ophthalmoscopy, pterygium surgery, tonography, vertical eye muscles, recent advances in ophthalmic research, ocular pathology, macula function, orthoptics, electrophysiology of the eye, and corneal transplants.

Additional information may be obtained by writing to Dr. John R. Finlay, Secretary, Alumni Association, 218 2d Ave., New York 3.

Seminar on Glaucoma.—A seminar on glaucoma, with particular emphasis on gonioscopy and the study of the anterior angle, will be given at the Brooklyn Eye and Ear Hospital on May 18-20, 1959. Ample opportunity for practical instruction in the use of the gonioprism will be given, and material from the glaucoma clinic will be utilized.

The course will be given by Dr. Daniel Kravitz, assisted by Drs. Mortimer A. Lasky, Nicholas P. Tantillo, and Samuel Zane.

Registration is limited to six ophthalmologists only.

Application and the fee of $50 may be addressed to Dr. Daniel Kravitz, Brooklyn Eye and Ear Hospital, 29 Greene Ave., Brooklyn 38.

Biological Photographic Meeting.—The first Midwestern Sectional Meeting of the Biological Photographic Association will take place here April 24 through 26 at the State University of Iowa, Iowa City. Distinguished photographic specialists in medicine, dentistry, biology, and other sciences will attend, representing major medical and dental schools, hospitals, research institutions, and science centers from Canada and nine midwestern states (Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, North and South Dakota, and Wisconsin).

A fascinating salon of color and black-and-white photographs will feature striking displays of clinical and specimen photography, photomicrography, and natural science. A motion picture program is scheduled for Friday evening, April 24, reviewing new medical and scientific films.

The Biological Photographic Association is the only professional society in the United States dedicated to the skills of medical and scientific photography. Founded at Yale University in 1931, the world-wide membership includes physicians, medical editors, educators in the fields of medicine and science, and staff photographers of medical and scientific institutions. The Association issues the authoritative *Journal of the Biological Photographic Association*, a quarterly pub-

lication of illustrated articles on scientific photography, motion pictures, and photomicrography in color and monochrome.

For further information write Mr. Carroll H. Weiss, Director of Professional and Press Publicity, 160 Riverside Dr., New York 24.

Meeting of the New Mexico Ophthalmological Society.—The 1959 Annual Meeting of the New Mexico Ophthalmological Society will be held in Santa Fe, N. Mex., April 30, May 1, and May 2, 1959. The guest speaker will be Dr. Peter C. Kronfeld. Among the topics to be discussed are "Complications of Cataract Surgery," "Current Techniques of Retinal Detachment Surgery," and "Diagnostic Problems." For further information, write Dr. J. A. Dillahunt, Secretary, 106 Girard Blvd., S. E., Albuquerque, N. Mex.

SOCIETY NEWS

Central Illinois Society of Ophthalmology and Otolaryngology.—The The newly elected officers of Central Illinois Society of Ophthalmology and Otolaryngology are as follows:

President: Dr. William F. Hubble, Decatur.
President-Elect: Dr. Charles D. Sneller, Peoria.
Vice-President: Dr. Edgar T. Blair, Springfield.
Delegate-at-Large: G. Leroy Porter, Urbana.
Secretary-Treasurer: Dr. Clarence A. Fleischli, Springfield.

The spring meeting will be a joint meeting with the Indiana Academy of Ophthalmology and Otolaryngology, French Lick, Ind., April 24-26, 1959. The fall meeting will be held in Decatur, Ill., Sept. 25 and 26, 1959.

International Council of Ophthalmology and International Federation of Ophthalmological Societies.—A meeting of the International Council of Ophthalmology was held during the XVIII International Congress of Ophthalmology in Brussels, on Sept. 6, 1958.

The following is a brief résumé of the more important business.

1. *Report of the Treasurer.*—The financial position of the Council was reported to be satisfactory. The contribution to the Council for International Organizations of Medical Sciences came under review. Hitherto the CIOMS received $100 U. S. annually from the Council and each International Congress paid $500 to the same body. In view of the increased subsidy desired by the CIOMS, the Council undertook to pay $300 U. S. annually and hoped to recoup itself to the extent of $500 from each International Congress. Great regret was expressed at the resignation of Amsler from the post of Treasurer, which he had filled for eight years with much distinction.

2. It was considered desirable to give more publicity to the Report on Ophthalmological Education throughout the world, presented to the Council in 1954. This report will be published in the *British Journal of Ophthalmology*, and a reprint will be sent to all universities with a Faculty of Medicine.

3. It was decided that the recently formed European Society of Ophthalmology would be represented on the International Council; François was accepted as the delegate. This society, which has been joined by all countries in Europe except Bulgaria and Russia, will hold its first congress in Athens in April, 1960.

4. The Index Ophthalmologicus (1958) was distributed gratuitously to all members attending the XVIII International Congress at Brussels. Further copies of this volume are obtainable through Dr. A. C. Copper (Coehoornsingel 42, Zutphen, Holland).

5. The Multilingual Ophthalmological Dictionary, published under the auspices of the Council, will be on sale early in 1959; the publisher is S. Karger AG., Basel.

A meeting of the International Federation of Ophthalmological Societies was held in Brussels on Sept. 8, 1958.

The recommendations of the International Council were in general approved. The following is a brief résumé of the other business.

1. It was decided that the subscriptions paid by affiliated societies should remain at the present figure of one-half Swiss franc per member per annum.

2. The new Statutes put forward in the International Congress of 1954, in Montreal were adopted with the modification that the national committee organizing the International Congress would have the right to refuse to accept communications submitted to it in order that the number of such communications could be reduced and their standard raised.

3. Two reports already passed by the International Council were accepted by the Federation. In the testing of the color sense in transport workers by isochromatic plates, the Swedish test, Boström (B II 1950) was admitted as well as the test of Ishihara. A report on visual safety on trunk roads concluded that trees bordering them or advertisements, if not too numerous, gave rise to no danger from the visual point of view.

4. The Ophthalmological Societies of Burma and Tunis were accepted as members of the International Federation.

5. It was decided that the XIX International Congress should be held in India at New Delhi in 1962.

6. *The New International Council.*—Office-bearers to the International Council and the replacements of 5 of the 10 ordinary members were elected to hold office during the next four years. The new Council consists of the following: Sir Stewart Duke-Elder, President; C. Berens, Vice-President; E. Hartmann, Secretary; B. Streiff, Treasurer.

Members: Arruga (Barcelona), Palomino Dena (Mexico), Espildora Luque (Santiago, Chile), Lyle (London), Melanowski (Warsaw), MacDonald (Toronto), Müller (Bonn), Paufique (Paris), Uyemura (Tokyo), Weve (Utrecht)

Ex-officio: Bietti (International Association for the Prevention of Blindness), Franceschetti (International Organization Against Trachoma), Payne (Pan-American Association of Ophthalmology), François (Pan-European Society of Ophthalmology), Coppez (President of the last Congress), The President of the next Congress, Copper (Secretary for the Index Ophthalmologicus).

Delta Gamma Foundation Scholarships.—Scholarships are being offered by the Delta Gamma Foundation for the training of orthoptic technicians, teachers of partially seeing children, and specialists for blind children. The deadline for applications is May 1, 1959. For further information, write the Delta Gamma Central Office, 1820 Northwest Blvd., Columbus 12, Ohio.

Postgraduate Courses at the Institute of Ophthalmology of the Americas.—The Institute of Ophthalmology of the Americas, New York Eye and Ear Infirmary, will offer the following postgraduate courses:

1. Ocular Surgery, May 18-29, 1959. It will include Cataracts; Keratectomies and Keratoplasties; Pterygium; Lacrimal Sac; Retinal Detachment; Muscles, including Ptosis; Glaucoma; Enucleation and Evisceration, and Orbitotomy. Registration is limited. Preference will be given to ophthalmologists taking the entire course. If places are available, doctors may register for individual parts.

2. Histopathology of the Eye, May 18-23, 1959. Registration is limited.

For further information, please write Mrs. Tamar Weber, Registrar, Institute of Ophthalmology of the Americas, New York Eye and Ear Infirmary, 218 2d Ave., New York 3.

Books

Highlights of Ophthalmology. By Benjamin F. Boyd. Price, $7. Pp. 281, including index, with 150 illustrations. Star & Herlad Co., Box 1189, Panama, R. P., 1958.

The three parts of this book are as follows: Part I covers the Pan-American Congress of 1957, the Chicago Ophthalmological Society Meeting of 1957, the Arthur Bedell Lecture of 1957, and a section on personal interviews. The latter seem to be tape recordings of interviews between the author and well-known ophthalmologists throughout the country. Part II is a new section on highlights of ocular surgery and includes the annual meeting on glaucoma sponsored by the National Society for the Prevention of Blindness. Part III covers the 1957 meeting of the American Academy of Ophthalmology and Otolaryngology, the last round-table discussion of the Society of Eye Surgeons and Eye Fitters, and another section on personal interviews.

The book is a curious collection of academic ophthalmology, textbook instruction at the first-year residency level, and news coverage with a slant toward the Time-Look-U. S. News style. The illustrations particularly fall into this pattern. While much that is contained in this little book can be criticized as being newspaper type of coverage, there is certainly a need to document what is going on in the ophthalmological world at the grass-roots level. In this respect, the editor is certainly to be congratulated.

Pathophysiology and Surgery. By James D. Hardy. Price, $19. Pp. 704, with 278 illustrations. The Williams & Wilkins Company, Mt. Royal & Guilford Aves., Baltimore 2, Md., 1958.

Occasionally one finds a book on general medicine or surgery which looks so interesting that a mental note is made to read it or at least peruse it at some future hoped-for leisure time. This is such a book. There is nothing in it bearing directly on ophthalmology, but there is much in it which would make one a better ophthalmic surgeon. For example, the chapters on the physiology of injury, including the local responses of the tissues; the general consideration of the water content of the body; the physiology of connective tissue and wound healing and the homotransplantation of tissues; the pathophysiology of thermal burns; radioactive isotopes; the biology of aging, and, finally, the physiology of anesthesia, are all parts of this book which are stimulating and look inviting. One functions better in small details if a broad base of general principles has been provided, and this is the type of book which provides these. Make sure that this book is in your department's ophthalmological library and find the few minutes necessary to broaden this base—it is rewarding.

Textbook of Physiology. By William D. Zoethout and W. W. Tuttle. Thirteenth edition. Price, not given. Pp. 712, with 305 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1958.

This very popular textbook of physiology has gone through thirteen editions. It has always had an excellent section on the physiology of the eye, and, for this reason, it should be well known to ophthalmologists. It is somewhat regrettable that in this edition the more recent advances in the physiology of vision have not been adequately dealt with. Instead of basic physiology half a page is devoted to ocular hygiene, which has no place in a book of this sort. It is to be hoped that in the next edition the section on physiology of the eye will be more adequately revised.

General Ophthalmology. By Daniel Vaughan, Robert Cook, and Taylor Asbury. Price, $4.50. Pp. 328. Lange Medical Publishers, Los Altos, Calif. 1958.

This book reviews the whole of ophthalmology for medical students, physicians, pediatricians, internists, and resident physicians in ophthalmology. It is really a

compendium, but, unlike most, the selection of the material has been very carefully done, and the book is ideal for reviewing ophthalmology for those about to be exposed to examinations. The illustrations are excellent.

One of the interesting features of the book is the announcement that the second edition will appear in 1960 and that the material for this has already been started. Although each subject is dealt with concisely, there is a good bibliography at the end of each chapter and the student may pursue his search for further information in this manner.

Mirror to Physiology: A Self-Survey of Physiological Science. By R. W. Gerard. Price, not given. Pp. 372. American Physiological Society, Inc., 9650 Wisconsin Ave., Washington 14, D. C., 1958.

This is a survey of the field of physiology and seeks to answer questions of interest to investigators, teachers, administrators, editors, and grantors who have a common interest in this branch of basic science. It represents an enormous expenditure of time and effort and will be of value to ophthalmologists interested in research in fields of ocular physiology, particularly if they are administrators. The author has viewed the results of his labors far too pessimistically when he states, "Fondly conceived as another Flexner report, it lacked the Flexner to produce it."

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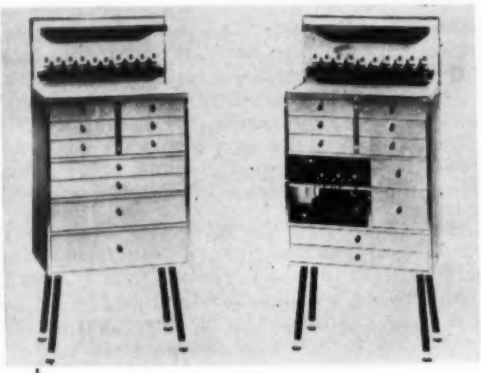

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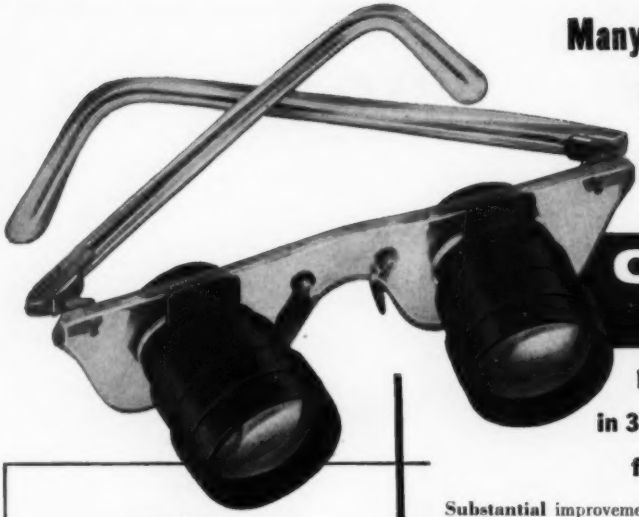

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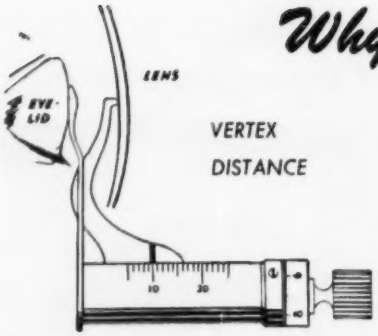

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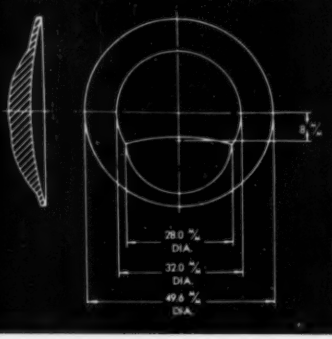

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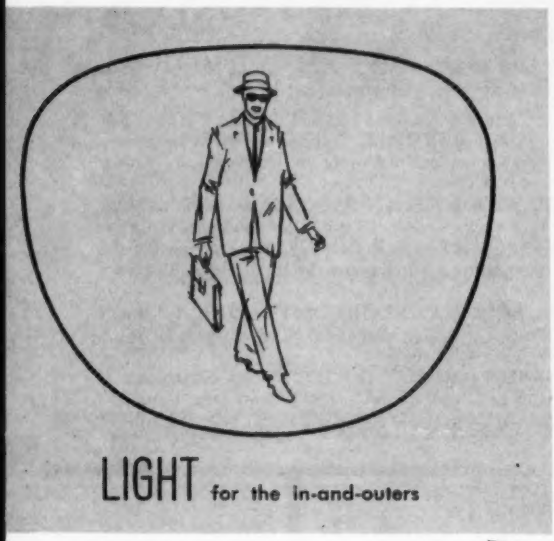

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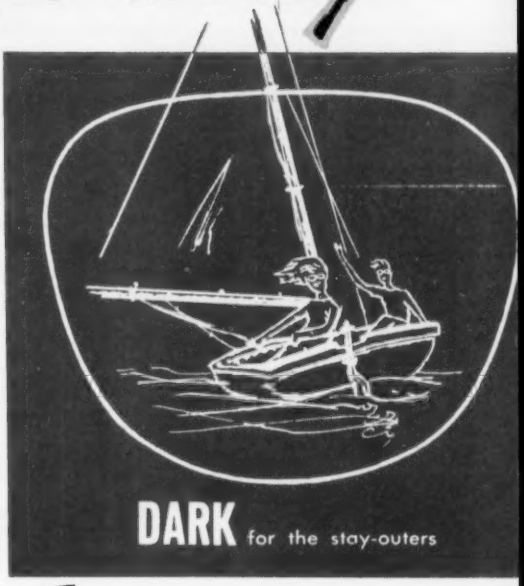

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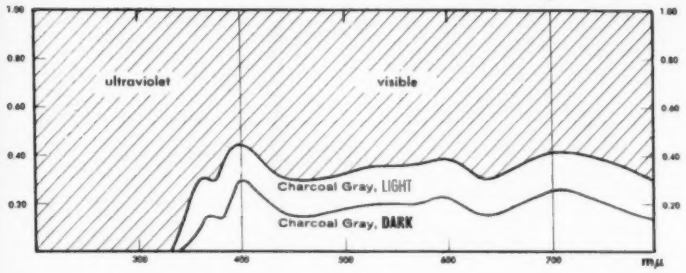

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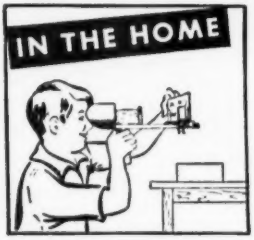

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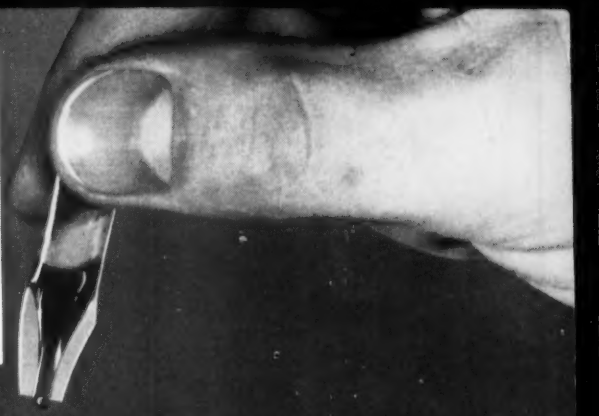

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